The Journal of Laboratory and Clinical Medicine

VOL. 22 JULY, 1937 No. 10

CLINICAL AND EXPERIMENTAL

AMEBIC DYSENTERY*

AN ANALYSIS OF THE LABORATORY DATA FROM 400 CASES

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RECENTLY, the authors[1] had occasion to make a clinical study of 400 cases of amebic dysentery admitted to the State Charity Hospital at New Orleans from August, 1928 to April, 1936. In all cases of this series *Endamoeba histolytica* was demonstrated either upon stool or proctoscopic examination, or both. All the patients had been admitted to the hospital because of symptoms directly attributable to this infection, and therefore no carrier cases were included in the series.

Since the literature shows few analyses of blood, gastric and roentgenologic findings in amebic dysentery, and these in relatively small series, the authors believe that such a study is worth while. Unfortunately, all 400 patients were not completely studied from a laboratory standpoint, but the available data are presented below. It must be emphasized that the findings occurred in the dysenteric form of amebiasis.

STOOL AND PROCTOSCOPIC EXAMINATIONS

The parasite was found in each of the 400 cases, either by examination of the feces alone, or in material obtained upon proctoscopy. Stool examination only was done in 261, and either cysts or trophozoites of *Endamoeba histolytica* were demonstrated in 212 cases (81.2 per cent of those examined). In the

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Received for publication, October 19, 1936.

vast majority of cases, a single stool examination was done for diagnostic purposes, although a number were also examined at the end of the course of therapy.

In 299 cases, proctoscopic examination was done with the demonstration of typical ulcers in 261 cases. Active trophozoites were found in 252 of these, or in 84.3 per cent of those examined. Clark[2] recorded the distribution of lesions at necropsy in 186 cases with 72.5 per cent of these presenting ulcers in the rectum. In the present series ulcers were found in 87 per cent of those examined with the proctoscope.

It, therefore, appears that, in cases of intestinal amebiasis presenting clinical manifestations, the diagnosis may be readily established in a high proportion of cases by a single examination of either the stool or rectum. No fair comparison can be made of the efficacy of either stool or proctoscopic examination in this series with those of other authors. At the time of examination, all the cases of the present series were showing clinical manifestations of intestinal amebiasis, and naturally the demonstration of amebae should be easier than in carrier cases. Brown,[3] in a series of over 500 cases, was able to find the organism in 68.2 per cent by single examination of the stool. Examination on three successive days assured finding it in 98 per cent of cases.

Multiple parasitism was recorded in a number of cases. Many of the fecal examinations were performed by the hospital internes who undoubtedly failed to record the presence of certain nonpathogenic parasites in some of the cases. *Endamoeba coli* was the organism occurring most commonly with *Endamoeba histolytica*. Table I lists the number of cases of multiple parasitism as well as the other parasites noted, with the frequency of their appearance.

TABLE I

OTHER PARASITES PRESENT		NUMBER OF CASES OF MULTIPLE PARASITISM	
Endamoeba coli	16	2 parasites present	34 cases
Endolimax nana	9	3 parasites present	4 cases
Iodamoeba butschlii	2	4 parasites present	4 cases
Giardia intestinalis	6	5 parasites present	0 cases
Trichomonas hominis	2	6 parasites present	1 case
Chilomastix mesnili	1		
Trichocephalus trichiurus	9		
Strongyloides stercoralis	6		
Ascaris lumbricoides	5		
Necator americanus	2		
Enterobius vermicularis	1		

BLOOD STUDIES

A study of the blood revealed several findings which were considered of interest.

A red blood cell count was made in 89 cases. The count was found to be below 4,500,000 in 64 per cent, or in 57 cases, and less than 4,000,000 in 39 of these. In some of the other cases in which the count was over 4,500,000, a secondary anemia may have been present but not obvious if frequent stools produced a concentration of the blood. Nine patients had a count of 5,000,000 or above, some of which may have resulted from dehydration. The hemoglobin values (generally estimated by the Tallqvist method) likewise showed the evi-

dences of secondary anemia. In 76 cases in which a hemoglobin value was recorded, 32 or 42 per cent had readings of below 70 per cent of hemoglobin. If 90 to 100 per cent is accepted as normal for the Tallqvist determination, 70 or 92 per cent of the cases had a hemoglobin of below normal level (Table II).

TABLE II

ERYTHROCYTE COUNT		HEMOGLOBIN	
CELLS	NO. OF CASES	PERCENTAGE	NO. OF CASES
1-1.9 million	2	40-49	4
2-2.9 million	7	50-59	12
3-3.9 million	30	60-69	16
4-4.4 million	18	70-79	19
4.5-5 million	23	80-89	20
Above 5 million	9	90 and above	6

That a secondary anemia should occur in clinical intestinal amebiasis is not surprising. Bloody diarrhea occurred in 313 of 400 cases. In some cases the loss of blood is not only appreciable in amount but, equally important, is often long continued, either one or both of which factors could well account for secondary anemia. Furthermore, in cases of long-continued diarrhea, even without loss of much blood, there may be nutritional imbalance due to incomplete digestion of food because of rapid peristalsis and, second, due to impaired absorption of the products of digestion, both of which can be a factor in the production of anemia.

In Craig's[4] experience, the leucocyte count averaged above 10,000 per c.mm. in uncomplicated cases, though he says some were below 8,000 and some as high as 20,000. Manson-Bahr and Willoughby[5] found a leucocytosis of 11,000 as an average in the study of 165 cases of intestinal amebiasis. Total white cell counts were recorded in 115 of the 400 cases studied. In 84 of these, the count was below 10,000 (75 per cent of cases). As may be seen in Table III many were below 7,500, and some few even below 5,000 cells per c.mm. Twenty or 17.3 per cent had a count only as high as 10,000 to 15,000 and in 11 cases the count was above 15,000. In 4 the count was above 20,000, the maximum being 29,000 white cells.

Craig[4] feels that a leucocyte count above 15,000 should lead one to suspect liver abscess. Among our 11 cases with a leucocytosis of over 15,000 there were 3 with proved abscess, and 2 with probable abscess (liver enlargement and x-ray signs). However, several of the abscess cases had counts below 12,000 and one even as low as 4,250 cells per c.mm. In the cases of our series which showed counts of over 15,000, the temperature ranged from 101° to 104° F. as a maximum in all but 3. Several of the high counts were in cases of dysentery of acute and severe onset.

Musgrave[6] found an increase of the granular cells in intestinal amebiasis. Manson-Bahr and Willoughby[5] reported an average of 57 per cent of polymorphonuclear cells in the cases of their series. In uncomplicated cases, Craig[4] noted an increase of polymorphonuclear cells more frequently than mononuclear cells. A differential white count was done in 105 of the 400 cases of this series (Table III). The majority, 73 or 69.5 per cent, had a polymorphonuclear cell

count of 75 per cent or below. Eighty-eight or 82 per cent showed lymphocytes of twenty or above, and of these 34 or 32.3 per cent of the total number had a lymphocytosis of above 30 per cent.

A study of eosinophiles has been carried out by several authors. Craig[4] feels that marked eosinophilia indicates the presence of other intestinal parasites, and that an increase of eosinophiles is not part of the picture of amebiasis. However, Manson-Bahr and Willoughby[5] found a maximum eosinophilia of 9 per cent in acute cases and 11 per cent in chronic cases. Musgrave[6] merely says there may be an increase, though of no diagnostic significance. Thirty-seven or 35.2 per cent of our cases had no eosinophiles present on examination of the blood. If we consider 2 per cent within normal limits, 33 or 31.4 per cent showed an eosinophilia, of which 15 cases were above 5 per cent. The maximum count was 16 per cent (Table III). Among the cases having eosinophilia were

TABLE III

WHITE CELL COUNT

TOTAL CELLS	NO. OF CASES	POLYMORPHONUCLEARS	NO. OF CASES
Less than 5,000	7	Less than 40%	2
5,000- 7,500	35	40-55%	12
7,600-10,000	42	56-75%	59
10,000-15,000	20	76-85%	23
Above 15,000	11	86% and above	9
LYMPHOCYTES	NO. OF CASES	EOSINOPHILES	NO. OF CASES
Less than 20%	19	0	37
20-30%	52	1- 2%	35
31-40%	22	3- 5%	18
41% and above	12	6-10%	9
		11-15%	5
		16% and above	1

several cases of additional parasitic infestation, as follows: 2 cases of *Ascaris* with eosinophilia of 8 and 5 per cent respectively; *Necator*, 1 case with eosinophiles of 12 per cent; and 4 cases of *Strongyloides*, with eosinophilia of 14, 8, 2, and 2 per cent, respectively.

GASTRIC ANALYSIS

Ten per cent, or 40 cases, of the series had had a gastric analysis (Table IV). The results of these analyses showed a condition not infrequently associated with a variety of diseases of the gastrointestinal tract, probably as a reflex

TABLE IV

GASTRIC ANALYSIS

DEGREES	TOTAL	FREE
0	1	13
1-10	5	6
11-20	5	6
21-30	4	4
31-40	7	6
41-50	6	2
51-60	7	3
61-70	1	-
71-80	3	-
81 and above	1	-

manifestation. This is the finding of either achlorhydria or hypochlorhydria in a relatively high proportion of cases. Thirteen or 32.5 per cent of cases showed an achlorhydria; another 16 or 40 per cent showed a free acid of 30 degrees or less. Thus 72.5 per cent of the 40 cases presented low values for free acid. Generally low values for total acidity were found, namely, 28 or 70 per cent were found to have only 50 degrees or less of total acid. As may be seen in the table, high acid values were uncommon. Since at times one finds indigestion due to low free acid, it might be well for clinicians to keep these findings in mind for it is not uncommon for achlorhydria or hypochlorhydria to persist for some time even though the etiologic factor in its production has been controlled.

ROENTGEN RAY STUDIES

Roentgen ray examination of the digestive tract was carried out in 31 of the 400 cases studied. This examination consisted of routine gastrointestinal series over a period of forty-eight hours, or of barium enema, or both. One exception to this was found in one case of right upper quadrant pain in which gallbladder visualization was carried out, and found to be negative. Gastrointestinal series and barium enema were both done in 4 of the 31 cases. Gastrointestinal study alone was carried out in 20 cases and barium enema alone in 6. The forty-eight-hour gastrointestinal examination was reported as negative in 11 of the cases and the barium enema as negative in 6 cases.

A variety of findings was reported in the group of cases accepted as showing an abnormality in those studied with use of barium by mouth. Regurgitation through the cardia into the esophagus was noted once, as was also a spastic pylorus in one case. Stasis in the terminal ileum occurred in one patient; in another was reported a dilated terminal ileum so distorted as to give the appearance of being looped about a mass in the right lower quadrant. The appendix was found filled at the forty-eight-hour study in 5 cases. Spasticity or irritability of the cecum occurred in 4 patients studied. The colon was found spastic in all segments in 5 and only in the ascending portion once. Stasis upon both twenty-four- and forty-eight-hour plates was noted coincidentally in cecum and ascending colon in 2 cases; it appeared alone in the ascending segment in one patient and in the transverse in another.

By use of the barium enema, stasis in the cecum was found twice. Ulcerative condition of the colon could be demonstrated in 4 cases with barium by rectum: in one the whole colon was involved; in the second, the descending segment; in the third, the sigmoid colon; and in the fourth, the rectum showed filling defects.

A correlation of the duration of symptoms with gastrointestinal x-ray findings led to no conclusions.

Apparently few conclusions are to be drawn from the roentgenologic gastrointestinal studies carried out in 31 cases of this series. Further, practically nothing is to be found in the literature on the subject. Ikeda[7] reported on the x-ray findings in the colon in 7 cases. His conclusions in the small series are not very helpful. He feels that the colon is negative in early amebic dysentery except for possibly some spasm. In the subacute or early chronic stage, the

spasticity is minimal, in light of the extent of the disease. His positive findings in the chronic stage were loss of flexibility of the colon wall, shortening and upward displacement of the cecum with local deformity, and patency of the ileocecal ring, allowing reflux into the terminal ileum. Vallarino[8] believes the diagnosis of amebic dysentery cannot be made by the roentgen ray, and that its value is mainly in determining the extent of the lesion. He found abnormalities most often in the region of the cecum, ascending colon, hepatic flexure and at times in the transverse colon.

In 27 of the cases of amebic dysentery, x-ray study was made of the diaphragm and lung bases, especially of the right side. Indications for obtaining such an examination in a given case were not clear from study of the patient's record. However, apparently the presence of a palpably enlarged liver was one good indication for further examination of the lung bases.

The diaphragm and lung bases were found negative in 13 cases, about 50 per cent, though in 4 of these the liver was easily palpable. Among the positive roentgen findings, the costophrenic angle, on lateral views, was found obliterated in 8 cases, 6 of the anterior, and 2 of the posterior angle. Six of the 8 presented liver enlargement on physical examination. In 4 other cases, the right leaf of the diaphragm was found to be elevated, and upon physical examination, 2 of these were found to have an enlarged liver. Diaphragmatic pleurisy of the right base, which may or may not be of significance, was reported in 2 further cases; one of these presented liver enlargement.

The importance of the roentgen ray in arriving at a diagnosis of amebiasis of the liver has been well brought out by Granger.[9] In addition to the abnormal elevation of the diaphragm as the most constant and principal x-ray sign of subdiaphragmatic pathology, he brings out further details related to liver abscess. He states that "elevation of the diaphragm with obliteration of the cardiophrenic angle in the posterior-anterior view and of the anterior costophrenic angle in the lateral view means subdiaphragmatic abscess secondary to a liver abscess."

CONCLUSIONS

1. In clinically active cases of intestinal amebiasis, the demonstration of *Endamoeba histolytica* is readily accomplished by either stool or proctoscopic examination.

2. A secondary anemia was present in the majority of cases. In some of the remaining cases, blood concentration due to dehydration may have masked this condition.

3. The leucocyte count was above 10,000 cells per c.mm. in only 26.9 per cent of the 115 cases in which it was determined.

4. The differential count showed no characteristic findings. The polymorphonuclear neutrophiles were above 75 per cent in 30 per cent of 105 cases. There was an eosinophilia of above 2 per cent in only about one-third of the cases in which a differential count was made.

5. Gastric analysis was done in 40 cases, of which 25 showed either an achlorhydria or definite hypochlorhydria.

6. Roentgen ray studies of the gastrointestinal tract were done in 31 cases. No definite conclusions can be drawn from such studies.

7. In 27 cases roentgenologic examination was made of the right diaphragm, which was found abnormal in 14 instances. Nine of these showed an enlarged palpable liver.

REFERENCES

1. Hinman, E. H., and Kampmeier, R. H.: Clinical Intestinal Amebiasis. A Study of 400 Cases From Charity Hospital at New Orleans, Am. J. Trop. Med. **17**: 263, 1937.
2. Clark, H. C.: The Distribution and Complications of Amebic Lesions Found in 186 Post-Mortem Examinations, Am. J. Trop. Med. **5**: 157, 1925.
3. Brown, P. W.: The Nature, Incidence and Treatment of Endamebiasis, J. A. M. A. **86**: 457, 1926.
4. Craig, C. F.: Amebiasis and Amebic Dysentery, Springfield, Ill., 1934, Arthur C. Thomas.
5. Manson-Bahr, P. H., and Willoughby, H. M.: On the Leucocyte Count in Intestinal Amoebiasis and Its Value as an Aid to Diagnosis, Tr. Roy. Soc. Trop. Med. & Hyg. **22**: 125, 1928.
6. Musgrave, W. E.: Amebiasis: A Clinical Study (Ed. by A. C. Reed), Am. J. Trop. Med. **11**: 469, 1931.
7. Ikeda, K.: Roentgenologic Observations of the Colon in Amebic Dysentery; Report of Seven Cases, Radiology **22**: 610, 1934.
8. Vallarino, J. J.: Report of Further Observations on the Value of Roentgen Rays in Estimating the Extent of Amebic Infection of the Large Intestine, Tr. Roy. Soc. Trop. Med. & Hyg. **22**: 209, 1928.
9. Granger, A.: Radiological Signs of Sub-diaphragmatic Abscess, New Orleans M. & S. J. **82**: 748, 1930.

THE RÔLE OF VITAMIN B_1 IN CARDIOVASCULAR DISEASES*

Preliminary Report

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IN 1927 and 1928 it was pointed out in the medical literature by one of us (B. S.) that the American diet is deficient in vitamin B_1[1] and that the deficiency of this dietary factor may be responsible for a large proportion of infant mortality, particularly since both breast[2] and cow's[3] milk are deficient in vitamin B_1, and because of the inefficiency of the lactating mother to secrete quantitatively this vitamin in the milk.[4] Clinical evidence of the beneficial results of vitamin B_1 in infant nutrition has since been presented by Hoobler,[5] Dennett,[6] Bloxsom,[7] Waring,[8] Bray,[9] Gaynor and Dennett,[10] Blatt and Schapiro,[11] Albert,[12] and others.

The existence of polyneuritis of pregnancy has been recently reported and the literature reviewed by Strauss and McDonald.[13] In their 1935 report on the Relation of Vitamin B Deficiency to Metabolic Disturbances during Pregnancy and Lactation, Tarr and McNeile[14] concluded that the requirements of

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Received for publication, October 16, 1936.

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this dietary essential during the gestation and lactation periods are much greater than for the nonpregnant and nonlactating woman, thus confirming the results on experimental animals.[2, 4, 15]

That vitamin B_1 is a complicating factor in malnutrition of the school child and that material benefits are derived, as manifested by growth, increase in height and vigor, by fortification of the diet of the school child with this vitamin, has been demonstrated by Morgan and Barry,[16] Summerfeldt,[17] and Ross and Summerfeldt.[18]

In 1932 Marks[19] reported that chronic vitamin B deficiency is of fairly frequent occurrence in medical practice, as manifested by a tendency to the development of chronic gastrointestinal disorders (anorexia, indigestion, constipation colitis) and the sequelae of such disorders, i.e., headache, "biliousness," skin eruptions, etc.); second, malnutrition, and impaired assimilation of food; third, impairment of strength, vigor, and resistance. He concluded that cereal germ (wheat embryo) is an efficient source of vitamin B for the treatment of such deficiencies.

In 1933 Grimm and coworkers[20] reported that tuberculous children improve on a dietary regime furnishing an increased supply of vitamin B_1 and minerals.

McClendon[21] claims that vitamin B deficiency is a complicating factor in acrodynia, and Rice[22] has observed beriberi symptoms in celiac disease.

Hutter and associates[23] are of the opinion that the atrophic tongue, which is a symptom-complex of pernicious anemia, is due to the complication of vitamin B deficiency in the primary anemia. Haden[24] recently pointed out that the complete treatment of pernicious anemia cannot be accomplished unless the diet is abundant in vitamin B_1 in addition to being satisfactory in other respects.

That the alcoholic addict's requirements for vitamin B_1 are unusually great has been recently demonstrated by Jolliffe and coworkers.[25]

The recent isolation of pure crystalline vitamin B_1[26] and its availability to the laboratory investigator and clinician having been made possible by the manufacturers,* promise to reveal much of interest concerning the physiology and human requirements of this essential dietary factor. The successful treatment of human beriberi with crystalline antineuritic vitamin has been reported by Hermano and Eubanas.[27] Recently, Vorhaus, Williams and Waterman[28] have presented evidence which suggests frequent deficiency of vitamin B_1 in the human dietary. In a study of 100 cases of clinical neuritis in which vitamin B_1 was administered orally in the form of 10 mg. daily of pure crystalline vitamin B_1 (Merck), 44 were rendered symptom-free, 48 were improved, and 8 showed no benefits. In a group of 8 cases of unexplained gastrointestinal hypotonicity and anorexia, 6 became free from all symptoms on ingestion of vitamin B_1; 2 improved.

In reviewing the literature relative to the advisability, rationality, and feasibility in the treatment of cardiac diseases with vitamin B_1, there was a striking resembance of the symptoms of cardiac disease and accentuated cases of vitamin B_1 deficiency. It is not unlikely that certain cardiac diseases may be

*Merck and Company, Rathaway, N. J.

due to accumulative effects of vitamin B_1 deficiency extending over a period of a number of years, as it is a known fact that the general American diet is deficient in vitamin B_1.

It was thought probable, since vitamin B_1 influences nerve tissue, that it would increase the neuromuscular system of the heart. In the group of patients where myocardial damage was more pronounced, these patients seemed to derive the greatest improvement. We did not confine our treatment to this type of patient, but included the arteriosclerotic heart and the hypertensive heart disease. Two types of cardiac diseases were excluded, syphilitic and rheumatic heart diseases.

In construction of the diet, the aim was to have one that was easily assimilated, producing as little work for the cardiovascular system as possible, yet furnishing sufficient food energy and high in vitamin B_1. The diet was supplemented by special cereals for breakfast, such as Pablum which contains yeast with large proportions of wheat embryo, and Ralston's whole wheat cereal which contained added wheat embryo. Brewers' yeast in malted milk and a provision of large quantities of fresh fruits and vegetables, which furnished the greater part of the menu for the evening meal, materially increased the vitamin B_1 intake of the cardiac cases. The number of patients under this special form of therapy was 30. In addition to this regime, 18 patients whose condition was more severe, received daily five pulvules of Lilly vitamin B_1 concentrate, furnished by the Eli Lilly Research Laboratories. Each pulvule contained 200 Sherman units, which added 1,000 units daily to their special diet. Twelve patients, whose condition was less severe, received 10 tablets of a commercial product, the potency of which is at the present time being determined by a biologic assay. The cardiac patients received approximately 1,500 to 2,200 vitamin B_1 units daily, which is from 5 to 7 times the amount contained in the average American diet. In addition, each patient received 2 glasses of orange juice daily and 10 drops of a saturated solution of potassium iodide. For precordial pain or discomfort, theamin capsules compounded with sodium amytal were given.

Table I is representative of the special diet given the cardiac patients for one week.

The average daily consumption of carbohydrates, 325 gm.; protein, 100 gm.; fats, 100 gm. The average daily caloric intake was 2,500 calories.

The classification of the patients was as follows: Arteriosclerotic heart disease, 14; hypertensive heart disease, 9; myocardial damage, 7; etiology undetermined.

In addition these patients had basal metabolism tests and all were within normal limits. Urinary excretion of vitamin C in 18 cardiac patients following saturation with orange juice.

In 1935 Harris and Ray[29] introduced a quantitative chemical method of determining vitamin C subnutrition in man by titrating daily excretion of this vitamin in the urine, using the dye, 2:6, dichlorophenolindophenol. They observed that normal adults on diets furnishing an adequacy of vitamin C excrete daily about 30 mg. of cevitamic acid. On diets low in vitamin C the daily

TABLE I

BREAKFAST	DINNER	SUPPER
Monday		
Applesauce	Purée split pea soup, crackers	Cream of purée beet soup, crackers
Pablum, cream	Broiled calf liver	Scrambled brains and eggs
Bacon, eggs	Buttered rice	Stewed tomatoes, croutons
Whole wheat toast, butter	Sliced tomato salad, mayonnaise	Head lettuce salad, mayonnaise
Coffee, milk, tea	Cottage pudding, whipped cream	Peaches in syrup
	Whole wheat bread, butter	Whole wheat bread, butter
	Tea, coffee, milk	Tea, coffee, milk
Tuesday		
Sliced bananas, cream	Barley soup, crackers	Cream of celery soup, crackers
Ralston's wheat	Baked spareribs, gravy	Baked lima beans, tomato sauce
Cereal, cream	Mashed potatoes	Buttered asparagus
Bacon, eggs	Buttered spinach	Combination fresh vegetable salad, mayonnaise
Whole wheat toast, butter	Waldorf salad, mayonnaise	Apricots in syrup
Coffee, milk, tea	Vanilla ice cream	Whole wheat bread, butter
	Whole wheat bread, butter	Tea, coffee, milk
	Tea, coffee, milk	
Wednesday		
Halves of grapefruit	Corn chowder, crackers	Purée split pea soup, crackers
Pablum, cream	Roasted leg of lamb, gravy	Creamed eggs on toast, points
Bacon, eggs	Candied sweet potatoes	Stewed cabbage
Whole wheat toast, butter	Buttered peas	Sliced tomato salad, mayonnaise
Tea, coffee, milk	Celery curls	Sliced pineapple
	Baked apples, cream	Whole wheat bread, butter
	Whole wheat bread, butter	Tea, coffee, milk
	Tea, coffee, milk	
Thursday		
Seedless grapes	Vermicelli soup, crackers	Cream of potato soup, crackers
Ralston's wheat	Meat loaf	Baked macaroni, cheese
Cereal, cream	Buttered rice	Buttered spinach
Bacon, eggs	Mashed turnips	Cardinal salad, mayonnaise
Whole wheat toast, butter	Sunset salad, mayonnaise	Peaches in syrup
Coffee, milk, tea	Baked apple betty, cream	Whole wheat bread, butter
	Whole wheat bread, butter	Tea, coffee, milk
	Tea, coffee, milk	
Friday		
Sliced oranges	Vegetable soup, crackers	Oyster stew, crackers
Pablum, cream	Baked catfish, lemon	Mashed browned potatoes
Bacon, eggs	Mashed potatoes	Buttered green beans
Whole wheat toast, butter	Blackeyed peas and salt pork	Buttered asparagus
Coffee, milk, tea	Celery curls	Apricot and peanut butter
	Blackberry blanc mange	Salad, mayonnaise
	Whole wheat bread, butter	Whole wheat bread, butter
	Tea, coffee, milk	Tea, coffee, milk
Saturday		
Crushed pineapple	Beef rice soup, crackers	Cream of lima bean soup, crackers
Ralston's wheat	Broiled steaks, gravy	Baked stuffed green peppers, gravy
Cereal, cream	Buttered noodles	Buttered diced carrots
Bacon, eggs	Cauliflower and milk sauce	Sliced tomato salad, mayonnaise
Whole wheat toast, butter	Head lettuce salad, mayonnaise	Blackberries
Coffee, milk, tea	Individual baked custards	Whole wheat bread, butter
	Whole wheat bread, butter	Tea, coffee, milk
	Tea, coffee, milk	
Sunday		
Halves of grapefruit	Cream of tomato soup, crackers	Cream of chicken soup, crackers
Pablum, cream	Roasted chicken, giblet gravy	Baked navy beans, tomato sauce
Bacon, eggs	Mashed potatoes	Buttered spinach
Whole wheat toast, butter	Broccoli and butter sauce	Buttered peas
Coffee, milk, tea	Celery curls	Peach and peanut butter
	Chocolate ice cream	Salad, mayonnaise
	Whole wheat bread, butter	Whole wheat bread, butter
	Tea, coffee, milk	Tea, coffee, milk

excretion is 11 to 20 mg. Following saturation with 500 to 600 mg. of cevitamic acid in the form of orange juice, a normal adult will spill over 80 per cent of the test dose in the urine within twenty-four hours, while an adult whose tissues are depleted of this vitamin, because his diet was low in vitamin C for a long period previous to the test period, will take five to six days before saturation is obtained. This work was substantiated by Abbasy, Harris, Ray and Marrack.[30]

Schroeder,[31] and Harde and associates,[32] using this test, reported that during fever vitamin C is used up more rapidly than it is normally, and hence larger doses are needed to restore depleted reserves of this vitamin. These findings were corroborated by Abbasy and coworkers.[30]

That there is a reduced excretion of vitamin C in pneumonia following saturation is indicated by the preliminary results of Harde, Rothstein and Ratish,[32] and in various types of purpura, metrorrhagias and acute lupus erythematosis, from the recent observations of Finkle.[33]

In their recent paper on Studies of Vitamin C Excretion and Saturation, Youmans and associates[34] state that a daily urinary excretion of 20 mg. of vitamin C and an excretion of 30 per cent of a test dose of about 600 mg. in adults is suggested as the lower limits of normal daily excretion and of "saturation" respectively. Hawley and coworkers,[35] however, found the lower limit of excretion of vitamin C of normal individuals following saturation to be 45 per cent of the test dose.

EXPERIMENTAL

Since our patients previous to the introduction of the high vitamin B diet, supplemented with vitamin B concentrates, were already on a satisfactory vitamin C diet (fortified with a glass of orange juice daily), the daily urinary excretion of vitamin C was much higher than reported by other investigators. It ranged between 32 to 81 mg. daily in 17 individuals and in one case was as high as 133 mg. Following saturation, however, for twelve to fourteen days with a daily intake of 4 glasses of undiluted orange juice, furnishing 500 mg. of cevitamic acid, the results were as follows:

Two patients excreted 63 per cent of the daily test dose of cevitamic acid; two, 47 to 53 per cent; one, 37 per cent; nine, 22 to 28 per cent; one, 18 per cent; one, 13 per cent; and two, 9 to 10 per cent. Thirteen of the eighteen patients under observation, or 72 per cent of the cases, excreted less than 30 per cent of the daily test dose of vitamin C, which judging from the experience of previous investigators, is an indication of abnormal vitamin C metabolism. Whether there is deficient absorption of vitamin C in cardiac or other diseases remains to be established.

The results submitted in this paper constitute a preliminary report. We feel, however, that the publication of our results at this time may be of interest to other investigators in the field of vitamins in clinical medicine.

SUMMARY

Clinically, these patients were easier to manage, were contented, and used less medication than any group we have had under our supervision at this

Facility. With the exception of three patients, all stated that they felt better than they had in years. No controls were used in this series, as our number of patients was rather limited. A new series is being started in which there will be controls. It is realized that we have not exhausted all the means of determining whether these patients have improved clinically, but the following report reveals that the patients have improved. Respiration average for the first week, of the 30 patients, was 21, the average for the last week under treatment was 18½. Pulse rate average for the first week was 89, the last week 79. Blood pressure showed definite improvement, with exception of one case, and the blood pressure gradually increased in this one instance. Patients had electrocardiograms before and after treatment, but changes were inconstant and no definite conclusion could be derived from them. The patients were walking from two to four miles per day at the time of discharge from this hospital, and reported no undue ill effects. This is definitely better than any group we have had under our supervision. It will be noted that their respiration and pulse rates and blood pressure showed improvement over admission to this hospital after indulging in exercise. Whether this improvement is entirely due to vitamin B_1 therapy or not we are unable to say. There were no deaths in this series of cases, and the average length of hospitalization was 114 days.

Thirteen of eighteen cardiac patients showed an abnormal vitamin C metabolism, indicated by the low urinary excretion of cevitamic acid following twelve to fourteen days' saturation with 500 mg. of a daily ingestion of cevitamic acid given in form of four glasses of orange juice daily.

We are indebted to the Clinical Laboratory of the Veterans Administration Facility, Fayetteville, Arkansas, for its cooperation.

REFERENCES

1. Sure, B.: The Unusual Response of Nursing Young (mus Norvegicus albinus) to Vitamin B Administrations, J. A. M. A. **89**: 675, 1927.
   Sure, B., and Schilling, S. J.: The Production of Beri-beri in the Nursing Young (mus Norvegicus albinus) Associated With Hemorrhages, Am. J. Dis. Child. **35**: 811, 1928.
2. Macy, I. C., Outhouse, J., Graham, A., and Long, M. L.: Human Milk Studies. The Quantitative Estimation of Vitamin B_1, J. Biol. Chem. **73**: 189, 1928.
3. MacLeod, F. L., Brodie, J. B., and MacLoon, E. R.: The Vitamin A, B (B_1) and G (B_2) Contents of Milk, J. Dairy Sc. **15**: 14, 1932.
4. Sure, B.: A Quantitative Biological Method for the Study of Vitamin B Requirements for Lactation, J. Biol. Chem. **76**: 673, 1928.
5. Hoobler, B. R.: Symptomatology of Vitamin B Deficiency in Infants, J. A. M. A. **91**: 307, 1928.
   Hoobler, B. R.: Use of Vitamin B Diets in Infants. Further Observations, J. A. M. A. **96**: 675, 1931.
6. Dennett, R. H.: Routine Use of the Vitamin B Factor in Infant Feeding, J. A. M. A. **92**: 769, 1929.
7. Bloxsom, A. P.: Vitamin B Requirements in Infancy, Am. J. Dis. Child. **37**: 1161, 1929.
8. Waring, J. I.: Beri-beri in Infants, Am. J. Dis. Child. **38**: 52, 1929.
9. Bray, G. W.: Vitamin B Deficiency in Infants; Its Possibility, Prevalence and Prophylaxis, Tr. Roy. Soc. Trop. Med. & Hyg. Trans. **22**: 9, 1928.
10. Gaynor, M. F., and Dennett, R. H.: Rice Polishings as a Source of Vitamin B Complex in Infant Feeding, J. Pediatrics **4**: 507, 1934.
11. Blatt, M. L., and Schapiro, I. E.: Influence of a Special Cereal Mixture on Infant Development, Am. J. Dis. Child. **50**: 324, 1935.
12. Albert, José: Studies on Infantile Beri-beri Based on Five Hundred Fourteen Cases, Rev. Filipina de med. y farm. **23**: 9, 1932.
13. Strauss, M. B., and McDonald, Wm. J.: Polyneuritis of Pregnancy, a Dietary Deficiency Disorder, J. A. M. A. **100**: 1320, 1933.

14. Tarr, E. M., and McNeile, O.: Relation of Vitamin B Deficiency to Metabolic Disturbances During Pregnancy and Lactation, Am. J. Obst. & Gynec. **29**: 811, 1935.
15. Evans, H. M., and Burr, G. O.: On the Amount of Vitamin B Required for Lactation, J. Biol. Chem. **76**: 263, 1928.
16. Morgan, A. F., and Barry, M. M.: Increased Growth Secured Through the Use of Wheat Germ, Am. J. Dis. Child. **39**: 935, 1930.
17. Summerfeldt, P.: The Value of an Increased Supply of Vitamin B_1 and Iron in the Diet of Children, Am. J. Dis. Child. **43**: 284, 1932.
18. Ross, J. R., and Summerfeldt, P.: Value of an Increased Supply of Vitamin B_1 and Iron in the Diet of Children, Part II, Am. J. Dis. Child. **49**: 1185, 1935.
19. Marks, H. E.: Chronic Vitamin B Deficiency, M. J. & Rec., March 2, 1932.
20. Grimm, P. D., Raphael, I. J., and Schnute, L. F.: Diet of Tuberculous and Non-Tuberculous Children, Am. J. Dis. Child. **46**: 751, 1933.
21. McClendon, S. J.: Acrodynia, a Vitamin Deficiency Disease, Arch. Pediat. **50**: 666, 1933.
McClendon, S. J.: Yeast and Irradiated Ergosterol in the Treatment of Acrodynia, J. A. M. A. **93**: 455, 1929.
22. Rice, C. V.: The Treatment of Celiac Disease From the Standpoint of Vitamin Deficiency, Arch. Pediat. **47**: 572, 1930.
Rice, C. V.: Celiac Disease Treated From the Standpoint of Vitamin Deficiency, Arch. Pediat. **50**: 358, 1933.
23. Hutter, A. M., Middleton, Wm. S., and Steenbock, H.: Vitamin B Deficiency and the Atrophic Tongue, J. A. M. A. **101**: 1305, 1933.
24. Haden, R. L.: The Complete Treatment of Pernicious Anemia, Am. J. Dig. Dis. & Nutr. **1**: 628, 1934.
25. Jolliffe, N., and Joffe, P. M.: Relation of Vitamin B (B_1) Intake to Neurological Changes in the Alcohol Addict, Proc. Soc. Exper. Biol. & Med. **32**: 1161, 1935.
Jolliffe, N., Colbert, C. N., and Joffe, P. M.: Observations on the Etiologic Relationship of Vitamin B (B_1) to Polyneuritis in the Alcohol Addict, Am. J. M. Sc. **191**: 515, 1936.
26. Williams, R. R.: Structure of Vitamin B.: J. Am. Chem. Soc. **57**: 229, 1935.
27. Hermano, A. J., and Eubanas, F.: The Treatment of Human Beri-beri With Crystalline Antineuritic Vitamins, Philippine J. Sc. **57**: 277, 1935.
28. Vorhaus, M. G., Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1: Experimental and Clinical Observations, J. A. M. A. **105**: 1580, 1935.
Vorhaus, M. G., Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1: Observations in Diabetes, Am. J. Dig. Dis. & Nutr. **2**: 541, 1935.
29. Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C Subnutrition by Urine Analysis **228**: 71, 1935.
30. Abbasy, M. A., Harris, L. J., Ray, S. N., and Marrack, J. R.: Diagnosis of Vitamin C Subnutrition by Urine Analysis. Lancet **229**: 1399, 1935.
31. Schroeder, H.: Die ausscheidung der ascorbin säure im gesunden und kranken Organismus, Klin. Wchnschr. **14**: 484, 1935.
32. Harde, E., Rothstein, I. A., and Ratish, H. D.: Urinary Excretion of Vitamin C in Pneumonia, Proc. Soc. Exper. Biol. & Med. **32**: 1088, 1935.
33. Finkle, P.: Observations on Excretion of Vitamin C in Some Vascular Diseases, Proc. Soc. Exper. Biol. & Med. **32**: 1163, 1935.
34. Youmans, J. B., Corlette, M. B., Akeroyd, J. H., and Frank, H.: Studies of Vitamin C Excretion and Saturation, Am. J. M. Sc. **191**: 319, 1936.
Corlette, M., Youmans, J. B., Akeroyd, J. H., and Frank, H.: A Clinical Study of Vitamin C Excretion, South. M. J. **29**: 37, 1936.
35. Hawley, E. E., Stephens, D. J., and Anderson, Geo.: The Excretion of Vitamin C in Normal Individuals Following a Comparable Quantitative Administration in the Form of Orange Juice, Cevitamic Acid by Mouth and Cevitamic Acid Intravenously, J. Nutr. **11**: 135, 1936.

THE CORRELATION BETWEEN RED CELL SEDIMENTATION RATE AND PLASMA PROTEINS*

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THE red cell sedimentation rate has assumed a considerable degree of importance in recent years as an aid in the prognosis of certain diseases, notably chronic arthritis and tuberculosis, and much work has been done in an attempt to elucidate its mechanism. The whole subject has recently been reviewed exhaustively by Reichel,[1] and no attempt will be made here to deal with the mass of literature which has accumulated.

As regards the factors influencing the sedimentation rate (S.R.) it has been generally accepted that an increased rate is associated with increase of fibrinogen or globulin and a reduced rate with increase of albumin or of the total volume of red cells. The following equations by which the S.R. can be calculated from the plasma constituents have been derived by Westergren, Theorell and Widström:[2]

$$\text{S.R.} = 140.4 \times \text{Fibrin.}\% + 6.22 \times \text{Glob.}\% - 6.09 \times \text{Alb.}\% - 25.5 \quad \text{(i)}$$

and by Bendien and Snapper.[3]

$$\text{S.R.} = \frac{45}{\text{Cell volume}} \times \text{Color Index } [12\ (\text{Fibrin. } \% - 3.5) + 2.5\ (\text{Glob. } \%\ 0\text{-}22)] \quad \text{(ii)}$$

Equation (i) is calculated from the partial correlation coefficients but (ii) is more or less empirical. In both cases the amounts of fibrinogen and globulin present in the plasma are used, though Westergren and others employ percentages and Bendien and Snapper grams per liter. The former also allow for albumin but not for cell volume, while the latter disregard albumin but take into account both the color index and the total volume of cells.

On the other hand, Aldred-Brown and Munro[4] were unable to find any parallelism between sedimentation rate and plasma proteins in their series of rheumatic cases. Unfortunately, they do not give the full details on which these conclusions are based.

Another set of data has recently been published (Davis[5]), and it seemed interesting to examine the degree to which these various series correspond. With this object in view, the figures of Bendien and Snapper and of Davis have been analyzed and compared with the results obtained by Westergren and others. The method of partial correlations has been employed, and it may not be out of place to describe briefly what the method entails.

Two characters or variables are said to be correlated when a change in one character is associated with a change in the other. The degree of association is expressed by the correlation coefficient r, which may have any fractional value between $+1.0$ and -1.0. A coefficient of unity indicates perfect correlation, and

*From the Wellcome Bureau of Scientific Research.
Received for publication, June 2, 1936.

this may be positive or negative. Lesser degrees of association are expressed by smaller fractions, positive or negative, and complete independence by zero. The variables to which the correlation coefficient refers are indicated by a subscript, thus r_{sf} means the correlation between S.R. and fibrinogen.

In practice it is often found that changes in the two variables to be studied occur in the presence of other quantities which also vary, so that direct treatment of the data obtained cannot be undertaken except in the relatively few cases where the other quantities remain constant. To avoid this waste of material, the method of partial correlations takes the variations of all the constituents into account, so allowing all the data to be used. The final result is obtained by eliminating successively the effects which can be ascribed to changes in the other constituents present. For example, the partial correlation between S.R. and globulin with the effects due to fibrinogen and albumin removed [written $r_{sg.fa}$*] is obtained by calculating directly from the data the correlations of zero order, i.e., r_{sg}, r_{sf}, r_{sa}, r_{gf} and r_{ga}. From these are derived the partial correlations of the first order, $r_{sg.f}$ etc.; finally by an extension of the process those of the second order, $r_{sg.fa}$, etc., are obtained.

In Table I are shown the partial correlation coefficients between sedimentation rate and fibrinogen, globulin and albumin as given by Westergren and others and as calculated from the data of Bendien and Snapper and of Davis.

TABLE I

	$r_{sf.ag}$	$r_{sg.af}$	$r_{sa.fg}$
Westergren	+0.81	+0.44	−0.36
Bendien	+0.82	+0.77	−0.33
Davis	+0.12	+0.42	−0.18

As regards Davis' series, the correlation between S.R. and globulin is significant, but the other two might have occurred as the result of chance. All the coefficients derived from Westergren's and from Bendien's data are significant as judged by the usual test (Fisher[6]).

The question is whether the differences in these correlations indicate real differences among the series from which they are obtained, or whether they can be considered as chance variations due to errors of sampling from a single population. This point can be decided by means of the z test (Fisher[6]), which shows that the correlations for S.R. and fibrin and S.R. and albumin as found by Westergren and for Bendien's data do not differ significantly from each but do differ significantly from those of Davis. Bendien's correlation between S.R. and globulin differs significantly from the other two which are themselves in agreement.

One must conclude therefore that these three series show real differences in the manner in which changes of sedimentation rate are associated with alterations in the amounts of the proteins present in the plasma. This may possibly be due to individual variations in the technic adopted by the different observers for fractionation of the plasma or to some other cause. Until this point is settled it would appear impossible to develop an equation by which the sedimentation rate can be predicted from the levels of the plasma proteins.

*Sedimentation rate, fibrinogen, globulin and albumin are represented respectively by s, f, g, and a. The dot between g and f indicates that the effects of f and a have been eliminated.

SUMMARY

The partial correlation coefficients between sedimentation rate and plasma proteins have been calculated for two series of data: those of Bendien and Snapper and of Davis. These correlations differ significantly from each other and also from those of Westergren, Theorell, and Widström.

REFERENCES

1. Reichel, H.: Blutkörperchensenkung, Vienna, 1936, J. Springer.
2. Westergren, A., Theorell, H., and Widström, G.: Plasmaeiweiss, Blutlipoide, Erythrocyten und Senkungsreaktion, Ztschr. f. d. ges. exper. Med. **75**: 668, 1931.
3. Bendien, W. M., and Snapper, I.: Zuzammenhang zwischen der senkungsgeschwindigkeit der roten Blutkörperchen und dem Eiweissspektrum, Biochem. Ztschr. **235**: 14, 1931.
4. Aldred-Brown, G. R. P., and Munro, J. M. H.: The Blood Sedimentation Rate and the Plasma Proteins, Lancet **1**: 1333, 1934.
5. Davis, J. S.: Protein Studies in Atrophic (Rheumatoid) and Hypertrophic Arthritis, J. LAB. & CLIN. MED. **21**: 478, 1936.
6. Fisher, R. A.: Statistical Methods for Research Workers, ed. 5, London, 1934, Oliver and Boyd.

PROLONGED RECTAL FEEDING*

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VERY little literature on rectal feeding (except for glucose) is available; the most important[3]: Loops were tied off of the colon and ileum and the absorption determined. Gelatin solution was practically not absorbed in the colon although the author concludes that 0.7 per cent sodium chloride increased the absorption of gelatin. Albumose solutions were hardly absorbed in the colon.

Bywaters and Short[2] gave amino acids by rectum, but the criterion of absorption was the increased elimination of nitrogen in the urine! A twenty-four-hour pancreas digest of milk as well as a sulphuric acid digest of protein was given and caused increased urinary nitrogen. Glucose was very well absorbed and delayed hunger. They recommend pancreas digest of milk with 5 per cent glucose added.

The Murphy drip was used for saline, peptonized milk, and 4 per cent glucose. The criterion of absorption was N balance. The nitrogen in the stools was also determined and the loss of body nitrogen was calculated to be an average of 13 grams per day. Only 3.9 grams could be supplied daily by rectal feeding and 2.06 grams were eliminated in the stools. Nothing was taken by mouth.[1]

EXPERIMENTAL

In a patient with ileocolostomy, in which it was finally determined that regurgitation from colon to ileum could occur, a prolonged rectal feeding was maintained for two months after intermittent trials for one month. During

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Received for publication, September 11, 1936.

the first twenty days, food was taken by mouth, but due to nausea, the patient ate less and less and practically ceased to eat about the thirtieth day, and practically all of the food was administered by rectum.

The rectal food was prepared in the following manner: In 900 c.c. of N/10 NaOH were dissolved the following amino acids in grams: 10 glutamic, 10 aspartic, 5 alanine, 1 tyrosine, 1 phenylalanine, 1 creatine, 1 serine, 1 valine, and 1 glycine; and in 900 c.c. of N/10 HCl there were dissolved 1 arginine, 1 lysine, 1 tryptophan, 1 histidine, 1 proline, 1 hydroxyproline, and 1 cystine. These were warmed until the amino acids dissolved and then mixed and 200 c.c. of a 95 per cent alcoholic solution of yeast-vitamin-Harris added, sufficient for ten days' requirement. Into this mixture was added 100 gm. of glucose. The results for the first 20 days are shown in Table I.

TABLE I

NITROGEN BY RECTUM	TOTAL N INTAKE	URINE NITROGEN	GAIN	LOSS
3.7	7.1	3.9	3.2	
3.24	5.6	6.2		0.6
	6.4	4.9	1.5	
	1.2	4.3		3.1
4.63	8.6	1.5	7.1	
	5.3	4.6	0.7	
	7.5	3.6	3.9	
	10.2	6.8	3.4	
4.16	13.5	9.9	3.6	
	10.4	5.9	4.5	
	7.5	4.5	3.0	
	9.4	5.7	3.7	
	6.2	3.6	2.6	
	6.4	4.2	2.2	
	6.9	4.2	2.7	
	6.1	4.2	1.9	
	5.3	4.2	1.1	
	4.2	4.4		0.2
	4.5	4.0	0.5	
	8.5	4.2	4.3	

During the last two months, the patient refused to eat more than an average of about 10 c.c. each of coffee, chicken soup, and eggnog, so that entire feeding had to be performed by rectum. At first the above amino acid mixture was tried and the patient was found to retain 400 c.c. per day. Then a casein hydrolysate was made by refluxing casein for twenty-four hours with 5 times its weight of constant boiling HCl, then evaporating off the HCl to near completeness, treating with decolorizing carbon and neutralizing with NaOH, making the NaCl content up to N/10 and the nitrogen equivalent up to 3.7 gm. in 400 c.c.

A study of this was made as follows: For several days the patient was given only water and at the end of this time was *losing 3.7 gm. nitrogen per day* and on the next day the patient was given 400 c.c. of rectal protein hydrolysate (3.7 gm. of nitrogen) and the *total eliminated was 3.9 gm. of nitrogen,* showing a very good utilization of the rectal nitrogen feeding. The patient was then continued for two months more on this feeding with the addition of 20 per cent alcohol, 5 per cent glucose, vitamin C and cod liver oil. It was

found that the patient could absorb 20 c.c. of cod liver oil per day, presumably by regurgitating it into the ileum. The main source of calories, however, was alcohol, and loss of weight was then rather small, in fact, only a pound a week as well as it could be determined by weighing the whole bed with the patient on it.

An attempt was made to give phosphates by mouth, and a solution containing 7 gm. of calcium and 13 gm. of phosphorus in the form of phosphoric acid and 0.1 gm. of iron was dissolved in a liter of water. The iron phosphate did not precipitate and it made a pleasant drink. However, 100 c.c. to supply Sherman's daily requirement could not be given. The patient would not take this much and finally refused it by mouth, in which case about one-tenth of Sherman's requirement was mixed in the rectal feeding.

Some of the tyrosine of the protein hydrolysate precipitated, but it was shaken up and remained in suspension during the rectal feeding. This method of feeding gives an opportunity of omitting lysine, for instance, in an attempt to inhibit the growth of cancer.

APPENDIX BY THE SENIOR AUTHOR

Since a little time is necessary for hydrolyzing a protein and removing the acid and humin, various sources of amino acids were investigated. Dr. Takeo Imai of the Tohoku Imperial University, Sendai, Japan, sent me some "aji-no-moto," which is said to be sodium glutamate. Sodium aspartate tastes the same, however, and it is not clear how a complete separation of the aspartate from the glutamate could be easily obtained. Ikeda decided that sodium glutamate was the basis of the taste of Oriental sauces. Buddhists are forbidden to kill, and they prepare vegetable sauces with the flavor of meat. The hydrolyzed casein which I prepared tasted similar to "aji-no-moto" and so did a sauce prepared from seaweed sent me by Dr. K. Oshima of the College of Fisheries at Hakodate, Japan. This latter contained 0.5 per cent iodine. Very many of these Oriental sauces, including soy sauce, which is also the basis of Worcestershire sauce, contain a variety of amino acids, and I tried some in rectal feeding.

REFERENCES

1. Adler, H.: Value of Rectal Feeding, Am. J. M. Sc. **150**: 562, 1915.
2. Bywaters, H. W., and Short, A. R.: Aminosäuren und Zucker bei der Rektalernährung, Arch. f. exper. Path. u. Pharmakol. **71**: 426, 1913.
3. Reach, F.: Untersuchungen über die Grösse der Resorption im Dick und Dünndarme, Arch. f. d. ges. Physiol. **86**: 247, 1901.

GASTRIC ACHYLIA*

Study of the Electrolytes of Gastric Juice

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Gamble and Mc Iver (1928) found that the gastric juice and blood serum are approximately isotonic. The chloride concentration in the gastric juice is somewhat greater than in the serum, but approximates that of the serum base. The acidity of the juice varies inversely with the fixed base which is combined with chloride to form neutral salts. This view has been substantiated by Bulger, Stroud and Heideman (1928). Studies by Bulger, Duff and Harrison (1925) on the arterial and venous blood to the stomach during secretion have shown that the chloride concentration remains the same, but the base of the venous blood exceeds that of the arterial. The chloride and water pass from the blood into the juice and leave behind the base which is neutralized by bicarbonate, making the blood more alkaline.

In the further analysis of the mechanism two views have arisen. Rosemann (1907), Delhougne (1930), Close (1929), and Duthrie (1930) envisage the secretion of the parietal cells as a mixed one consisting of hydrochloric and neutral chlorides. As the parietal cells become active the concentration of neutral chlorides decreases and that of hydrochloric acid increases. Hollander (1934) has concluded that parietal secretion is essentially an isotonic solution of hydrochloric acid. Mixed gastric juice would then consist of the secretion of parietal cells to which has been added another secretion containing pepsin, mucus, proteins, and other organic constituents. This secretion may be the result of both transudation of lymph and active secretion from other gastric and mucus-forming cells.

The present contribution is a study of the secretion of gastric juice in patients who do not secrete free HCl (achylia gastrica), and who presumably have a depression or inhibition of parietal activity.

METHODS

The gastric juice was collected by aspiration through a Rehfuss tube at fifteen-minute intervals over a period of ninety minutes. This constituted a control experiment without histamine stimulation. A second experiment was made in which 0.1 mg. histamine per 10 Kilo body weight was injected sub-

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Received for publication, November 19, 1936.

cutaneously immediately after the passage of the tube. The procedure otherwise was the same. This constituted an experiment with histamine stimulation. Later in the work the procedure was modified as follows:

Preliminary (7 A.M. to 12:30 P.M.)

7 A.M. Routine ward breakfast served.

8:30 A.M., 9:30, 10:30, and 12:30, 200 c.c. water was taken by mouth, total 800 c.c.

12:30 bladder emptied, urine discarded.

Control Period (12:30 to 2:00 P.M.)

2 P.M. Urine secreted since 12:30 P.M. voided and saved for examination.

Venous blood withdrawn for chemistry.

Experimental Period (2:00 P.M. to 3:30 P.M.)

2:00 P.M. Rehfuss tube passed, stomach aspirated at 30-, 60-, and 90-minute intervals. Histamine was either given or not, depending on the character of the experiment.

3:30 P.M. Venous blood withdrawn for chemistry. Bladder emptied and urine saved for examination.

Gastric Juice.—The total base was determined by Stadie and Ross method (1925), chlorides by Van Slyke (1923), nitrogen, phosphorus and pH by Hawk and Bergeim (1931), acidity by titration with dimethyl amino azobenzene and phenolphthalein as indicators, Na, K, and Ca by Peters and Van Slyke (1932).

TABLE I

QUANTITY SECRETION OF GASTRIC JUICE*

ACHYLIAS			INTERMEDIARY			CONTROL		
PATIENT	NO HISTAMINE	HISTAMINE	PATIENT	NO HISTAMINE	HISTAMINE	PATIENT	NO HISTAMINE	HISTAMINE
3	24.0	50.0	2	24.5	38.0	1	110.0	102.0
6	13.0	27.0	5	18.0	38.0	10	42.0	332.0
8	44.0	31.5	7	28.0	215.0	12	22.0	188.5
9	76.0	36.0	11	73.0	103.0	16	24.5	38.0
13	42.0	40.0	18	20.5	79.5	17	49.0	109.0
14	24.0	33.0				19	20.5	143.5
15	26.5	23.5				20	35.5	102.5
26	20.5	24.5				21	46.5	119.0
27	35.0	26.5				22	111.0	150.0
28	65.0	60.0				23	87.0	188.0
29	23.5	20.0				24	20.5	33.5
30	16.0	11.0						
31	27.0	48.0						

*Values are given in cubic centimeters over ninety-minute collection.

Blood Serum.—The total base was estimated by Stadie and Ross method (1925) and chloride by Van Slyke (1923).

Urine.—The chlorides were determined by Van Slyke (1923), ammonia and pH by Hawk and Bergeim (1931), base by Stadie and Ross (1925), titratable acidity by Folin (1903). Wherever the analytic values would change with time, the analyses were made at once. The other analyses were completed within twenty-four hours. The patients were all hospitalized at the time of the experiment. There were a total of thirteen patients with achylia, five patients in whom no free acid was found until after the administration of histamine and eleven patients with free acid present before the administration of histamine.

Results With Achylia After Histamine Stimulation.—The subcutaneous injection of histamine produces a watery secretion of high acidity in normal pa-

tients. This occurs in spite of an occasional fall in blood pressure, in which systolic readings of 80 to 65 were noted, and the presence of profuse sweating. An increase in quantity was found in the intermediary and control cases. In the achylias no increase was found.

Acid Values.—No "free acid" was found in any sample from patients with achylia gastrica, either before or after the stimulation with histamine. This is an accepted test for the diagnosis of achylia. Table II shows that the "combined acid" values are low and are not affected by histamine stimulation. The average acid value of 71 samples of gastric juice was 6.6 m.eq. per liter. The individual variations from this value were not large.

TABLE II

BASE CHLORIDE VALUES, ACHYLIAS (M. EQ. PER LITER)

PATIENT	GASTRIC ASPIRATION IN MINUTES	NO HISTAMINE				HISTAMINE			
		COMB. ACID	BASE	CHLORIDE	NITROGEN	COMB. ACID	BASE	CHLORIDE	NITROGEN
3	30	6.0	62.0	57.0	80.2	5.0	42.0	43.0	48.2
	60	6.0	36.0	33.0	32.1	5.0	40.0	52.0	64.2
	90	5.0	49.0	45.0	60.0	6.0	47.0	48.0	53.2
6	30	7.0	115.0	100.0	86.4	5.0	87.0	90.0	77.4
	60	6.0	117.0	105.0	86.4	5.0	114.0	102.0	77.4
	90	6.0				6.0	113.0	102.0	86.0
8	30	7.0		45.0	34.4	11.0	79.0	55.0	24.1
	60	11.0		64.0	50.8	17.0	66.0	40.0	31.1
	90	7.0	99.0	65.0	33.3	20.0	73.0	42.0	
9	30	20.0	72.0	38.0	23.3	5.0	84.0	60.0	25.1
	60	20.0	75.0	48.0	35.4	17.0	88.0	61.0	32.1
	90	35.0	63.0	41.0	30.1	4.0	88.0	58.0	16.9
13	30	5.0	94.0	67.0	30.1	21.0	132.0	98.0	35.7
	60					4.0	133.0	105.0	120.5
	90	3.0	119.0	89.0	74.2	10.0	126.0	101.0	33.3
14	30	4.0	72.0	31.0	16.1	3.0	149.0	31.0	18.3
	60	2.0	64.0	30.0	16.6	3.0	163.0	30.0	30.6
	90	2.0		39.0	25.7	3.0	151.0	32.0	26.8
15	30	4.0				5.0	66.0	18.0	18.9
	60	3.0				4.0	64.0	74.0	20.1
	90	3.0				4.0	55.0	31.0	14.9
26	30		73.7	49.3	46.0	2.0	77.5	52.4	64.2
	60		79.7	68.5			85.5	71.0	80.4
	90	3.0	78.2	63.3	60.0	4.0	81.0	60.3	40.2
27	30	4.0	55.0	43.0	66.9	4.4	54.5	47.5	42.8
	60	4.0	52.5	37.0	80.3		76.5	66.0	
	90	3.8	57.5	37.0	96.4	3.8	63.0	56.5	33.8
28	30	9.4	87.0	67.0	32.1	15.0	85.0	64.0	40.2
	60	4.6	78.0	60.0	40.2	14.0	74.0	62.0	74.3
	90	5.0	79.0	61.0	48.2	9.0	77.4	60.0	53.5
29	30	3.4	111.0	94.0	74.2	5.0	74.0	75.0	64.2
	60	2.8	78.0	70.0	53.3	4.0	48.0	55.0	28.3
	90	3.2	58.0	46.0	32.1	4.2	53.4	58.0	48.2
30	30		62.0	51.0		3.0	99.0	74.0	
	60	3.0	42.0	33.0	49.3	3.0	94.0	70.0	
	90		124.0	107.0		6.0	103.0	110.0	
31	30	4.0	92.0	85.0	59.5	2.0	128.0	115.0	
	60	4.0	75.0	84.0	48.0	2.0	127.0	104.0	
	90	4.0	122.0	85.0	48.0	2.0	138.0	107.0	

Average: Combined Acid, 6.6 m.eq. per liter. Base, 81.3. Chloride, 62.9. Nitrogen, 47.6.

Comparison of Base and Chloride Values to Those in Serum.—It may be seen (Table II) that the average chloride value for 73 estimations was 62.9 m.eq. (per liter) and that the average base value for 70 estimations was 81.3 m.eq. (per liter). In a few instances only do they approximate those found in the serum (chlorides, 110 m.eq. and base, 167 m.eq.).

The data of Bulger, Stroud, and Heideman (1928) show the same relationship.

Martin (1932 and 1934) found in cases showing no free acid, the chloride value of 33 to 137 m.eq. with a majority value of 80. The base ranged between 90 and 140 m.eq. with average values of 113. In these instances also the values are below those found in the serum.

Comparison of the Sum of Base and Acid Values to Chloride.—On the basis of theories of acid secretion to which attention has been called, the base values recede as the acid increases and increase as the acid diminishes. Hence, a study of the relationship of base plus acid to chloride seemed important. Table II shows that the average value of base plus acid is 81.3 m.eq. plus 6.6 m.eq. or 87.9 m.eq. per liter. The average value of chloride is 62.9 m.eq. The magnitude of the base plus acid excess over chloride is 25 m.eq., a quantity which by virtue of its size must be significant.

In Table III it will be noted that out of sixty-four estimations of base plus acid and chloride values in patients with achylia 58 showed an excess of base plus acid over chlorides. In the intermediary and control cases the base plus acid excess over chlorides occurred in 44 cases and chloride excess in 42 cases. The scatter on either side of equivalence was identical. The great majority of the individual determinations on either side did not vary from equivalence over 1.5 to 2.5 equivalents. In other words as long as the patient has an acid mechanism which is capable of stimulation (intermediary and controls), the sum of acid plus base is equivalent to chlorides. When the patient loses this mechanism (achylia) there is a preponderance of base. The corollary follows that all of the base is not bound to chloride.

TABLE III

SUMMARY OF RELATIONSHIP OF BASE PLUS ACID TO CHLORIDES IN GASTRIC JUICE

TYPES OF CASES	BASE PLUS ACID EXCESS		BASE PLUS ACID = CHLORIDE		CHLORIDE EXCESS	
	NO HISTAMINE	HISTAMINE	NO HISTAMINE	HISTAMINE	NO HISTAMINE	HISTAMINE
Achylias	26	32		2	3	1
Intermediary	8	9			7	5
Control	14	13	2	8	15	15

pH Values.—The range of the pH of the gastric juice can be seen from Table IV. These findings agree with results previously reported by Streicher (1930) that neutral red when injected intravenously into patients with no "free acid" does not appear as the red dye in the gastric juice. It will be recalled that neutral red develops its color in a pH of 6.8.

Nitrogen Values.—Table II shows the nitrogen values of gastric juice expressed in milliequivalents of nitrogen. These values are somewhat higher than

TABLE IV

pH OF GASTRIC JUICE IN ACHYLIAS

DIAGNOSIS AND CASE NUMBER	HISTAMINE STIMULATION		
	30 MINUTES	60 MINUTES	90 MINUTES
Pernicious anemia No. 13	6.8	7.0	7.2
Pernicious anemia No. 28	6.5	7.0	
Pernicious anemia No. 30	6.5	6.8	
Pernicious anemia No. 32	6.6	6.7	7.2
Alcohol gastritis No. 31	7.0	7.2	7.0

those reported in stomachs actively secreting HCl by Polland and Bloomfield (1929) and Martin (1934) where the dilution factors would evidently be active. They are also higher than those reported by Martin (1932) and Polland and Bloomfield (1929) for achylia gastrica cases. Since there is little or no volume change in the juice from patients with achylia gastrica under stimulation, these variations are doubtless not significant.

Urinary Changes.—Urine collections were made in four periods. In each experiment, it was collected for one and one-half hours before the stomach tube was swallowed. After the tube was inserted, urine was collected for another hour and a half, either following the injection of histamine or no injection. The urinary Cl, total base, titrable acidity, ammonia, and pH were determined. It was expected that in the control cases there would be evidence of a secretion of a more alkaline urine; in the intermediary cases, it was expected that a urine of less alkalinity would be excreted; and that in achylia gastrica cases, there would be no significant change in urinary secretion.

The data show that there are no significant changes in the pH after the injection of histamine. The study of the other urinary constituents likewise failed to show any definite evidence of excretion of a more alkaline urine. Doubtless the amount of acid juice removed from the stomach was too small to induce measurable changes in the body fluids. Since there was no acid removed in achylias, no changes would be expected.

Blood Studies.—Studies of base and chlorides of the blood showed no constant change after the injection of histamine, and for this reason a tabulation of their values is omitted. Again this is attributed to relative small quantity of gastric juice lost in comparison to the total body fluids from which the gastric juice was drawn.

DISCUSSION

As previously noted, gastric juice in achylia is a fluid which contains an amount of chloride and base distinctly below the values found in blood or lymph. The quantity of the juice is not influenced by the injection of histamine, which is a powerful vasodilator, and should increase the lymph flow. For these reasons it would seem safe to say that the gastric juice in achylia does not represent lymph seepage.

The secretion of the acid components of gastric juice is attributed to the parietal cells. A search of the literature on morphologic studies (Evans, 1926, Delafield and Prudden, 1907) of patients suffering from pernicious anemia and

achylia gastrica shows an atrophic gastritis and a destruction of the parietal cells with a transition of the chief cells from columnar to cuboidal shapes. In addition there is found inflammatory and degenerative reactions. More recent studies (Baker, 1936) in gastritis show that the degenerative process may extend to atrophy of both chief and parietal cells.

In the absence of parietal cells one would not expect HCl secretion. However, one might assume that the parietal cells have not been destroyed, but have been damaged and are secreting a juice of lower acid content corresponding to the "combined acid." If the acid mechanism were at all active some of the cells when working at a maximal capacity under the "all or none law" should secrete acid of fairly high concentration. One would expect therefore to occasionally find sporadic cases or instances in which the juice would show "free acid." A patient undergoing a remission might be expected to have free acid and during a relapse to have none. In other words, transition cases should be found. The long list of studies in the literature, the experiments with neutral red and our own studies are a unit in showing that such examples are not found.

The inference would seem to be justified that the "combined acid" which may be titrated in cases of achylia gastrica must be explained on some other basis than a neutralization of HCl by the buffer action of protein. For within the limits of the pH of gastric juice from patients with achylia (6.5 to 7.2), the protein acts as a weak acid and consequently would not form a protein HCl salt. If one searches for salts that may be titrated in the pH zone found in achylia gastrica, he is at once brought to the consideration of the $BHCO_3$. Martin (1934) has noted in one case of a benign achylia, values of HCO_3 ions varying from 17.5 to 30 m.eq. per liter. In a case of pernicious anemia values ranging from 4.7 to 5.7 m.eq. per liter were reported. No other determinations have been found. The studies of Gamble (1922) on carbonic acid and bicarbonate in the urine are of interest in this connection. If one assumes that the true values for HCO_3 ions are the same in the stomach as those in the blood, one would have a condition analogous to that noted by Gamble in the urine. The quantity of HCO_3 would be stationary and the quantity of bicarbonate in the gastric juice would then vary inversely as the pH. With a pH in the juice of 6.5 to 7.2, the milliequivalent of $BHCO_3$ would range from 5.6 to 21.6 m.eq. By reference to Table II it will be noted that the values of combined acid average 6.6 m.eq. per liter with a high value of 35 m.eq. The agreement between those values for "combined acidity" and the expected values for $BHCO_3$ within the ranges of the pH found in juices from achylia patients would indicate that one might be titrating $BHCO_3$ when he is titrating "combined acid."

At this pH the proteins of the gastric juice also would be acting as weak acids and these could be titrated and contribute a component, even though it might be a small one, to the "combined acidity."

The base in the juice of gastric achylias exceeds the chlorides. This base would be envisaged as combined with HCO_3 to form $NaHCO_3$ and with protein to form base proteinate salts. The chloride which is present represents neutral salts as NaCl.

From the above discussion it would seem that the parietal cell mechanism is not active in patients suffering from achylia and forms no secretion.

In the normal individual the protein salt secretion which dilutes and neutralizes the HCl and contributes enzymes and proteins to the juice, is usually attributed to the chief cells located in the fundus. From the literature the pepsin is much decreased or absent in patients with achylia. It is possible therefore that the juice obtained from such patients may represent a much-reduced output from chief cells of the fundus or it may arise from the pyloric glands. The evidence available does not permit a decision.

CONCLUSION

1. The quantity of gastric secretion in patients with achylia gastrica is not increased by histamine (0.1 mg. per 10 Kilo body weight).

2. The average chloride and base values of the achylia juices were distinctly below the values of these electrolytes in the serum. The values of base and combined acid exceeded those of chlorides. The values of base alone exceeded those of chlorides. The pH varied between 6.5 and 7.2. The nitrogen values showed individual variations and no significance was attached to these variations.

3. In patients with normal and intermediary acid secretion under the stimulation of histamine the juice contained an amount of base plus acid equal to the amount of chlorides.

4. There was no evidence of a secretion of a more alkaline urine in either controls or intermediary cases or those with achylia gastrica after the injection of histamine. The amount of juice aspirated in the first two classes of cases was too small in comparison with total available body fluids to affect urinary excretion. No change would be expected in patients with achylia gastrica.

5. There were no constant changes in total base and chlorides in the serum after the injection of histamine in any one of the three classes of cases.

6. The gastric juice in cases with achylia gastrica probably arises from the chief cells of the fundus or from the pyloric glands. The value for combined acid is doubtless due to the titration of bicarbonate and proteins. There is no evidence of any residual activity of the parietal cells.

REFERENCES

Gamble, J. L.: Carbonic Acid and Bicarbonate in Urine, J. Biol. Chem. **51**: 295, 1922.

Bulger, H. A., Allen, D., and Harrison, L. B.: Studies of Chemical Mechanism of Hydrochloric Acid Secretion; Observation on Blood Passing Through Stomach of Dogs, J. Clin. Investigation **5**: 561, 1928.

Bulger, H. A., Stroud, C. M., and Heideman, M. L.: Studies of Chemical Mechanics of HCl Secretion; Electrolyte Variations in Human Gastric Juice, J. Clin. Investigation **5**: 547, 1928.

Hollander, F.: Studies in Gastric Secretion; Composition of Gastric Juice as Function of Its Acidity, J. Biol. Chem. **104**: 33, 1934.

Rosemann, R.: Beiträge zur Physiologie der Verdaunng. 1. Die Eigenschaften und die Zusammensetzurg des durch Scheinfutterung gewonnenen Hundemagensaftes, Arch. f. d. ges. Physiol. **118**: 467, 1907.

Delhougne, F.: Stomach Secretion; Acid and Sodium Chloride Concentration in Gastric Juice, Deutsche Arch. f. klin. Med. **23**: 447, 1930.

Cowgill, G. R.: A Contribution to the Study of the Relation Between Vitamin B and the Nutrition of the Dog, Am. J. Physiol. **57**: 420, 1921.

Stadie, W. C., and Ross, E. C.: Microscopic Method for Determination of Base in Blood and Serum and Other Biological Materials, J. Biol. Chem. **45**: 735, 1925.
Van Slyke, D. D.: Determination of Chlorides in Blood and Tissues, J. Biol. Chem. **58**: 523, 1923.
Hawk and Bergeim: Practical Physiological Chemistry, ed. 10, Philadelphia, 1931, P. Blakiston's Son & Co.
Peters and Van Slyke: Quantitative Clinical Chemistry. Volume II: Methods, Baltimore, 1932, Williams and Wilkins.
Martin, L.: Gastric Secretion—Electrolytes Before and Their Changes at Various Periods After Histamine Stimulation, Ann. Int. Med. **6**: 91, 1932.
Martin, L.: Acid-Base Balance of Gastric Juice, Blood and Urine Before and at Intervals After Stimulation of Gastric Juice by Histamine, Bull. Johns Hopkins Hosp. **55**: 57, 1934.
Streicher, M. H.: Gastric Achylia; Differential Diagnosis With Aid of Neutral Red, J. A. M. A. **95**: 326, 1930.
Polland, W. Scott, and Bloomfield, Arthur L.: Quantitative Measurements of Pepsin in Gastric Juice Before and After Histamine Stimulation, J. Clin. Investigation **7**: 57, 1929.
Evans, Frank A.: Pernicious Anemia, Baltimore, 1926, Williams and Wilkins, p. 53.
Gamble, J. L.: Carbonic Acid and Bicarbonate in Urine, J. Biol. Chem. **51**: 295, 1922.
Crohn, B. B.: Affections of the Stomach, Philadelphia, 1927, W. B. Saunders Co., p. 376.
Delafield, F., and Prudden, P. M.: A Textbook of Pathology, ed. 8, New York, 1907, W. Wood & Co., p. 609.
Duthrie, R. J.: Action of Histamine on Chloride Content of Stomach, Quart. J. Med. **23**: 447, 1930.
Baker, N.: Histopathology of Gastritis, Mayo Clinic Bulletin, Aug. 12, 1936.

SECOND ATTACK OF MENINGOCOCCIC MENINGITIS WITH RECOVERY*

Two Cases

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IN COMMON with the other infectious diseases, one attack of meningococcic meningitis does not confer complete immunity in all cases. In addition to the infrequent occurrence of "a marked persistence of the meningococcus in the patient, apparently cured, and not sufficiently immunized by the first attack,"[1] there is the possibility of new infections. Some French writers consider this possibility very rare; others, of varying schools of thought, believe it to occur in as high as 20 per cent of cases.[2-5] The consideration of this potentiality increases the physician's responsibility, both before and after the initial infection.

The story of relapse is one in which there may be a low-grade active chronic infection with more than one active phase until recovery or death, or there may be intervals of complete quiescence between periods of marked activity each of which can be recognized as acute meningitis. As high as thirteen episodes have been noted.[4] The group with which we are dealing is that in which the patient is apparently recovered, showing no manifestations of infection, and having the disease again at a later date. The infective agent may be from without or

*From the Philadelphia General Hospital.
Received for publication, November 21, 1936.

within (Flexner). The occurrence of any form "implies incontestably that the immunity in such cases had not yet been obtained or had disappeared" (Nettier).[3]

When serum is used in treatment, the mortality rate drops and the relapse rate increases. On the other hand, when serum is not used, the mortality rate is very high and the relapse rate is very low.[6] Despite the advances made with serum, there remains an immunologic loophole through which a small number of patients are liable to repeated infections. This is the problem of prophylaxis. Perhaps the development and perfection of a specific antitoxin which is the latest advance will permit of adequate measures of active protection to bolster methods of treatment. The indictment written in 1898 that "there is little . . . bearing on the subject of immunity in this disease" is still true to a degree.[7]

A *relapse* is a state of renewed activity from the same underlying infection. Aaser reported a case of hydrocephalus in which the meningococci were found in the cerebrospinal fluid over two years after the original infection.[8] There have been reports of meningitis following trauma in which organisms were activated in injured tissues. A *fresh infection* or second attack obviously is a repetition of the whole disease syndrome possibly modified by the previous infection and treatment but having no bacteriologic link with the previous attack. The dividing line is difficult to establish. For practical purposes a patient can be considered as recovered when, while ambulant, examination of the cerebrospinal fluid shows a normal number of cells, persistent negative spinal fluid culture under lowered oxygen tension, normal spinal fluid sugar and chloride values, normal temperature and pulse, and absence of abnormal neurologic signs.

In 1884, Marchifava and Wright described the meningococcus. Three years later Weichselbaum showed the relationship of these organisms to cerebrospinal meningitis. In the eleven years until 1898, Councilman, Mallory, and Wright could find only five cases in the literature in which "the individual is reported to have had the disease twice, and in one of these the second attack is to be considered a relapse and not a fresh infection."[9]

The following is the report of two cases of second infection in patients considered recovered from the first infection.

CASE 1.—On Aug. 28, 1935, the police brought to the hospital a twenty-eight-year-old, well-nourished Italian, male, who was stuporous, tossing restlessly in restraints. Two days before admission he had suffered frontal headache which was not relieved by simple medication. The following morning the headache was generalized and persistent. Shortly after getting out of bed he had marked nausea and vomiting after which he collapsed and recalled nothing until after having been in the hospital for several days.

The personal medical history was not relevant.

On admission he was acutely ill, toxic, stuporous. His pulse was noted to be relatively slow. The skin was hot, dry, and clear. There was a marked cyanosis of lips and fingers. He lay on his side, head thrown back, knees drawn up. His pupils were widely dilated, round, not reacting to light. There was marked nuchal rigidity and bilateral Kernig sign. The spinal fluid was under pressure of 55 mm. of mercury. It was cloudy and examination showed 1,900 cells of which 90 per cent were of the polymorphonuclear variety, and the

remainder, lymphocytes; the sugar value was 15 mg. and chlorides 647. Smear revealed organisms morphologically meningococci which was confirmed by culture. The strain of organisms was not determined.

During the next two days he was given 215 c.c. of standard nonconcentrated anti-meningococcic serum. The first dose of 25 c.c. was given intraspinally; the second, of 30 c.c. intravenously well-diluted in normal saline solution; and the third 25 c.c. intramuscularly. Succeeding doses were given by all three routes. Repeated spinal taps showed the fluid to be progressively clearer. Occasionally 15 to 25 c.c. of air were injected intraspinally. By the third day he showed marked improvement. Each day 75 to 90 c.c. of spinal fluid were removed and by the eleventh day the fluid was clear, under normal pressure. The cell count was 40, sugar 44 and chlorides 702. During the next thirty days he was clinically well but had a very slight occasional elevation of temperature which became normal a month later. For the next month he was ambulant and was discharged in good condition. Blood, nose, throat, and spinal fluid studies were negative.

On Nov. 14, 1935, he was brought to the hospital in the same condition as on his first admission. It was found later that he had resumed his usual activities and lived in the same abode. Two days before this admission he had a severe headache followed by nausea, vomiting, and collapse. The spinal fluid showed 4,000 cells of which 88 per cent were polymorphonuclear, sugar was 30, and chlorides were 692. The organisms were found by smear and confirmed by culture to be meningococci. The patient was sensitive to serum, and he was desensitized after which he was given 90 c.c. of serum during the next two days, for the most part, intraspinally. Four days after admission he was much improved but still had some nuchal rigidity and bilateral Kernig sign. On the sixth day he had a serum reaction which was controlled by usual measures. The next day his temperature was normal and remained flat. Daily spinal taps were done and two weeks later the fluid showed 10 cells, sugar 49, and chlorides 738. During the following month a weekly spinal tap was done and the examination remained persistently negative. He was discharged apparently well seven weeks after admission; spinal fluid showed a normal number of cells, spinal fluid culture under lowered oxygen tension was negative, normal spinal fluid sugar and chloride values, normal temperature and pulse, and absence of abnormal neurologic signs.

CASE 2.—*(From Dr. Riesman's Service.)* On June 20, 1935, a well-nourished negro of twenty-eight was admitted in the same condition as that described for the previous patient. In addition he had a draining sinus on his neck. Spinal fluid contained over 2,000 cells of which 90 per cent were polymorphonuclear. Meningococci were demonstrated by smear and culture. He was given 450 c.c. of the nonconcentrated serum by the three routes, but the organisms could be found in the spinal fluid for several days after the institution of the treatment. He slowly improved and by July 1 was clinically well. Daily taps were done with the removal of large amounts of fluid and on July 10 he was ambulant. His Kahn test was strongly positive. Repeated studies of blood, nose, throat, and spinal fluid were negative, and he was discharged on July 19.

On Jan. 15, 1936, this same patient was re-admitted, semistuporous, having nuchal rigidity, inequality of pupils, bilateral Brudzinski sign, and bilateral Kernig sign. The spinal fluid showed 1,500 cells; meningococci were shown by smear and culture. During the next two days 70 c.c. of the concentrated serum were given by the three routes and by the third day the patient was alert. An additional 40 c.c. of serum were given, and he had daily taps with progressively clearer fluid. All the studies remained negative. The spinal fluid was three-plus in the cholesterol antigen. On February 10 the patient was discharged in good condition.

CONCLUSIONS

There is no diagnostic procedure at present to determine when a patient who has had meningococcic meningitis has completely recovered not only from the disease but also from the infection. Consequently, one cannot differentiate sharply relapse from fresh infection. A criterion has been suggested as an

approximation of the distinction. Newer immunologic agents may yield opportunities for certainty, and active immunization may lower the incidence of second infections as well as primary infections.

SUMMARY

1. Since it is not possible at this time to be certain when an infection with the meningococcus is ended, patients who have had the disease must be regarded as potentially liable to additional attacks.

2. Successive infections are not uncommon but complete case reports in the literature are few in number.

3. We present two cases of which the second attack of the disease was proved each time. The patients fulfilled the criteria proposed before discharge.

We are indebted to Dr. Riesman for his permission to report the second case and for his kind cooperation.

REFERENCES

1. Remond, A., and Colombies, H.: Cerebrospinal Meningitis Recurring After an Eight Months Interval, Bull. et mém. Soc. méd. d. hôp. de Paris **49**: 737, 1925.
2. Foster and Gaskell: Cerebrospinal Fever, 1916, Cambridge University Press.
3. Nettier, A.: Delayed Recurrence in Cerebrospinal Meningitis, Bull. et mém. Soc. méd. d. hôp. de Paris **42**: 527, 1918.
4. Sophian, A.: Epidemic Cerebrospinal Meningitis, London, 1913, Henry Kimpton.
5. Herrick, W. W.: Chapter XII in "Modern Medicine" of Osler, W., and McCrae, T. **1**: 567, 1925.
6. Ritchie, J.: Relapses in Cerebrospinal Fever, Edin. M. J. **1**: 505, 1910.
7. Councilman, W. T., Mallory, F. B., and Wright, J. H.: Cerebro-spinal Meningitis and Its Relation to Other Forms of Meningitis, J. Bost. M. Sc. **2**: 53, 1897-1898.
8. Aaser, E.: Case of Hydrocephalus With Gram-Negative Diplococci in the Cerebrospinal Fluid Two and One-Half Years After an Attack of Cerebrospinal Meningitis, Tidsskr. f. d. norske Laegefor, Køhenh. **25**: 607, 1915.
9. Councilman, W. T., Mallory, F. B., and Wright, J. H.: Am. J. M. Sc. **115**: 251, 1898.

THE NORMAL URINARY IODINE OF MAN*

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THE true picture of iodine metabolism cannot be determined by investigation of the total blood iodine alone. This determines principally the amount of mobile iodine. It does not indicate its destiny. It reveals nothing of its origin, utilization, storage or excretion.[20] It has, therefore, become increasingly apparent that it is necessary to determine the urinary excretion of iodine in order better to interpret clinical data concerning the thyroid gland and its relation to iodine metabolism. Consequently, we report an extensive investigation of the daily urinary excretion of iodine of normal individuals living in a region (Columbus, Ohio) of moderate endemic goiter. In addition, this paper presents a comparison of our investigation with similar studies made in five nongoitrous and in four other goitrous regions. We also discuss certain normal variables and fluctuations in the excretion of iodine by the kidney.

LITERATURE

Von Fellenberg,[1] in 1924, was one of the first to report the daily urinary iodine loss of normal individuals. He made a determination on each of seven normal subjects from the slightly goitrous region of Effingen in Northern Switzerland. He found a daily variation of from 28 to 108 micrograms,† with an average of 64. He has also recorded an extensive study made upon himself.[2] The latter has already been reviewed.[3] In addition he investigated the urinary iodine excretion of individuals in two other goiter-free regions. One, in Forte dei Marmi on the Ligurian coast of Italy,[4] where the daily variation in excretion was from 30 to 140 micrograms with an average of 72; the other, in Vik i Sogn, Norway,[5] where he found a range of excretion of from 94 to 240 micrograms per day with an average of 146. However, these[1, 2, 4, 5] twenty-four-hour excretions were calculated from determinations made on nocturnal urines. He based this procedure upon the assumption that the urinary excretion of iodine per hour is approximately constant. He had drawn this conclusion from his previous work.

The iodine content of water in Danzig, a coastal city, was found to be 10 micrograms per liter,[6] which is unusually high. This value is more than three hundred times the iodine content of the water in Berne, Switzerland, which

*From the Department of Research Surgery of The Ohio State University.

Received for publication, November 21, 1936.

A part of this work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Read before the Ninth Annual Meeting of the Central Society for Clinical Research at Chicago, Illinois, November 6, 1936.

†A *microgram* is one thousandth of a milligram (0.001 mg.). It is also designated a gamma.

von Fellenberg investigated. Liek,[6] in 1927, determined the urinary iodine of healthy men in Danzig. He observed a daily range of from 200 to 590 micrograms with an average of 343.

Lunde,[7] in 1928, reported a determination made on each of twenty-five nongoitrous, healthy, full-grown men who abstained from eating seafood on the day before the collection of the urine and, so far as was known, during the week previous to the day of investigation. These individuals lived in the Sandsvaer goiter district of Norway where the goiter incidence varied from 30 per cent to 60 per cent. The daily urinary iodine excretion varied from 6 to 83 micrograms with an average of 38. These values as those of von Fellenberg were calculated from nocturnal urines.

Scheringer,[8] in 1931, made 29 determinations on 17 normal nonpregnant women in Berlin. These ranged from 66 to 389 micrograms per day with an average of 141. A determination on each of 7 normal men varied from 103 to 214 micrograms daily and averaged 173.

McCarrison and his associates[9] reported analyses of single specimens of urine from men in Coonor in Northern India where there is no endemic goiter. They found from 26 to 72 micrograms of iodine per liter. Nongoitrous persons in Gilgit, also in Northern India, had a range of from 3 to 34 micrograms per liter in the spring[9] and from 1 to 22 micrograms per liter in the late summer and fall.[10] These determinations are of lesser value since the twenty-four-hour urinary volume was not given.

In Pécs, Hungary, the average daily iodine intake was calculated by Scheffer[11] as about 110 micrograms per day. In 1931 to 1932, Scheffer[12] made a determination of the urinary iodine on each of five individuals. These data revealed a range of daily excretion of from 21 to 33 micrograms with an average of 27. Scheffer[12, 13] has also made iodine balance experiments on normal individuals. These have been reviewed.[3]

Szász[54] has reported the urinary iodine of normal men living in high altitudes.

Elmer and Scheps[14] made urinary iodine determinations on two normal subjects in Lwów, Poland. The daily urinary iodine was 34 micrograms (average of 3 days) and 40 micrograms (average of two days), respectively.

This review of the literature (Table II) reveals that the normal urinary excretion of iodine has been previously investigated in any one individual for only short periods; never continuing over four days and usually for one day or less. Consequently, we considered it important to investigate the normal urinary excretion of iodine for periods of considerably longer duration.

This report is concerned with an extensive investigation of the normal urinary iodine of thirteen individuals without evidence of thyroid disease.

METHODS

The patients were maintained on a diet which was well balanced and as attractive as possible. Foods known to have a high iodine content, particularly seafoods, were excluded.[15] No thyroid substance nor iodine in any form was administered.

Collection of the twenty-four-hour urine specimens was made by trained attendants and was carefully supervised. The majority of the analyses of the blood and urinary iodine were made by the Phillips and Curtis[16] method. Others were made by Matthews' modification[17] of the Leipert[18, 19] wet-ashing method. The latter is simpler, more rapid, applicable clinically, and its results compare favorably with those obtained by the usual dry-ashing methods.[16]

OBSERVATIONS

The ages of these thirteen patients (Table I) varied from eight to fifty-nine years. Four were female and nine were male. They revealed no evidence of thyroid disease nor of other endocrine disorder. They presented no abnormality, such as evidence of kidney damage, which might so far as is known affect iodine metabolism. Six subjects (Table I, Subjects 5, 6, 7, 9, 10 and 13) were essentially normal individuals. The other seven presented varying diseases.

CASE 1.—R. V. (No. 330316) was a white boy of eight years with a *congenital harelip and cleft palate.* The cleft palate was surgically closed seven days previous to the commencement of this study. He was slightly febrile during this investigation. Otherwise, his recovery was uneventful.

The blood iodine on Apr. 29, 1933, was 9.5 mcg. per cent, which is normal. The urinary iodine concentration was 8.4 mcg. per cent (Table I, 1), which is less than that of the blood iodine. The daily urinary iodine excretion, over a period of ten days (Table I, 1), averaged 41 mcg., with the low and high limits at 10 and 125 mcg., respectively.

CASE 2.—J. T. (No. 331743) was a white male of thirty years with a *draining pleural sinus* resulting from chronic empyema. He was hospitalized one week previous to this study. The temperature was slightly subnormal throughout.

The blood iodine on Apr. 27, 1933, was 10.9 mcg. per cent, which is normal. The urinary iodine concentration was 6.0 mcg. per cent (Table I, 2), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of fifteen days (Table I, 2), averaged 58 mcg. with the low and high limits at 7 and 162 mcg., respectively.

CASE 3.—G. F. (No. 331105) was a colored male of fifty-nine years with *arteriosclerotic gangrene* of the left foot. His great toe was amputated on Mar. 8, 1933. Six weeks of succeeding hospital bed rest preceded this study. He was afebrile during its course.

The blood iodine on May 27, 1933, was 9.3 mcg. per cent, which is normal. The urinary iodine concentration was 5.6 mcg. per cent (Table I, 3), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of twenty-two days (Table I, 3), averaged 78 mcg. with the low and high limits at 29 and 196 mcg., respectively.

CASE 4.—G. W. (No. 331757) was a white female of thirty-two years with an old *traumatic deformity* of the right foot. A plastic operation on the ankle had been performed five days previous to the beginning of this study. Convalescence was uneventful. The blood Wassermann reaction was positive. Kidney function as tested was normal. The basal metabolic rate on May 17, 1933, was minus 6 per cent, with the basal temperature 98.2° F., pulse 64, respirations 20, and the weight 167 pounds.

The blood iodine on May 12, 1933, was 12.5 mcg. per cent, which is normal. The urinary iodine concentration was 5.5 mcg. per cent (Table I, 4), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of thirty-one days (Table I, 4), averaged 36 mcg. with the low and high limits at 11 and 135 mcg., respectively.

CASE 5.—N. S. was a *normal white female* of twenty-seven years who volunteered for this investigation. No disease was present. The basal metabolic rate on May 19, 1933, was minus 6 per cent, with the basal pulse 70, temperature 98° F., respirations 16, blood pressure 115/86, and the weight 131 pounds.

TABLE I

NORMAL DAILY URINARY EXCRETION OF IODINE OF MAN IN CENTRAL OHIO

SUBJECT NO.	DATE	SUBJECT		SEX	AGE	DAYS OF INVESTIGATION	AVERAGE 24-HOUR URINARY VOLUME C.C.	AVERAGE IODINE CONCENTRATION MCG. %	LOW AND HIGH LIMITS OF DAILY URINARY IODINE EXCRETION MCG.*	AVERAGE 24-HOUR URINARY IODINE MCG.
1	Apr., 1933	R. V.,	No. 330316	Male	8	10	465	8.4	10-125	41
2	Apr.-May, 1933	J. T.,	No. 331743	Male	30	15	950	6.0	7-162	58
3	Apr.-May, 1933	G. F.,	No. 331105	Male	59	22	1,445	5.6	29-196	78
4	Apr.-May, 1933	G. W.,	No. 331757	Female	32	31	740	5.5	11-135	36
5	Apr.-May, 1933		N.S.	Female	27	35	890	7.2	22-142	57
6	Dec., 1933	E. S.,	No. 336247	Male	28	15	990	4.9	36- 63	48
7A	Jan.-Feb., 1934	M. K.,	No. 340175	Female	14	29	1,720	2.9	19-130	47
7B	Apr.-May, 1934	M. K.,	No. 340175	Female	14	14	1,250	4.3	29- 76	48
8	Aug., 1934	P. B.,	No. 343967	Male	30	17	1,410	3.5	26-110	48
9	Aug., 1935	R. G.,	No. 354130	Female	26	4	470	9.2	25- 59	43
10	Oct., 1935	C. S.	No. 355691	Male	34	8	850	6.8	17-104	59
11A	Dec., 1935	H. B.,	No. 355729	Male	30	3	510	7.9	26- 68	41
11B	Jan., 1936	H. B.,	No. 355729	Male	30	11	640	7.2	17- 86	45
12	July, 1936	D. C.,	No. 363406	Male	8	5	1,560	2.7	32- 57	41
13	July, 1936	C. L.,	No. 363527	Male	54	8	1,180	5.8	31-145	68
Grand Average							1,005	5.9		51
Total Number of Days of Investigation						227				
Low and High Limits of Daily Urinary Iodine Excretion									7-196	36-78

*A microgram (mcg.) equals 0.001 mg., equals a *gamma*.

The blood iodine on Apr. 27, 1933, was 9.0 mcg. per cent, which is normal. The urinary iodine concentration was 7.2 mcg. per cent (Table I, 5), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of thirty-five days (Table I, 5), averaged 57 mcg. with the low and high limits at 22 and 142 mcg., respectively.

CASE 6.—E. S. (No. 336247) was a white single male, aged twenty-eight years, and a taxicab driver who volunteered as *a normal subject* for calcium and iodine balance investigation.[3, 20] The basal metabolic rate on Dec. 3, 1933, was minus 1, with the basal pulse 59, temperature 97.6° F., respirations 12, blood pressure 100/64, and the weight 121 pounds.

Three blood iodine determinations averaged 13.4 mcg. per cent, which is normal. The urinary iodine concentration was 4.9 mcg. per cent, which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of fifteen days (Table I, 6), averaged 48 mcg. with the low and high limits 36 and 63 mcg., respectively.

CASE 7.—M. K. (No. 340175) was a white girl of fourteen years with an *idiopathic scoliosis* of the thoracic spine. She was otherwise normal. The first study, which began on Jan. 12, 1934, was made during a preliminary period of hospitalization. A plaster shell was applied on Feb. 8, 1934. The second less extensive study which began on April 28, 1934, was made while she was being prepared for a spinal fusion.

The blood iodine on Jan. 22, 1934, was 14.0 mcg. per cent, which is normal. The daily urinary excretion of iodine (first study), over a period of twenty-nine days (Table I, 7*A*), averaged 47 mcg. with the low and high limits at 19 and 130 mcg., respectively. The urinary iodine concentration was 2.9 mcg. per cent, which is less than that of the blood iodine.

The blood iodine on Apr. 30, 1934, was 12.4 mcg. per cent, which is normal. The urinary iodine concentration (second study) was 4.3 mcg. per cent (Table I, 7*B*), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of fourteen days (Table I, 7B), averaged 48 mcg. with the low and high limits 29 and 76 mcg., respectively.

CASE 8.—P. B. (No. 343967) was a white male of thirty years with a *chronic pyogenic osteomyelitis* of the right ilium and the right femur head. There had been a previous staphylococcus pyarthrosis. During this study he was afebrile.

The blood iodine on Aug. 11, 1934, was 9.5 mcg. per cent, which is normal. The urinary iodine concentration was 3.5 mcg. per cent (Table I, 8), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of seventeen days (Table I, 8), averaged 48 mcg. with the high and low limits at 26 and 110 mcg., respectively.

CASE 9.—R. G. (No. 354130) was a white female of twenty-six years, who was markedly *obese,* weighing 243 pounds. She was otherwise quite normal. She presented no evidence of endocrine disturbance. Two basal metabolic rate determinations averaged minus 9 per cent.

The blood iodine on Aug. 5, 1935, was normal. The urinary iodine concentration was 9.2 mcg. per cent (Table I, 9). The daily urinary excretion of iodine, over a period of four days (Table I, 9), averaged 43 mcg. with the low and high limits at 25 and 59 mcg., respectively.

CASE 10.—C. S. (No. 355691) was a white male of thirty-four years who was a *psychoneurotic* of the neurasthenic type with anxiety neurosis. Ten basal metabolic rate determinations averaged minus 9 per cent.

The blood iodine on Oct. 22, 1935, was 10.4 mcg. per cent, which is normal. The urinary iodine concentration was 6.8 mcg. per cent (Table I, 10), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of eight days (Table I, 10), averaged 59 mcg. with the low and high limits 17 and 104 mcg., respectively.

CASE 11.—H. B. (No. 355729) was a white male of thirty years with *arrested pulmonary tuberculosis* after a right apical thoracoplasty on July 4, 1935. During the present study he also was being prepared for the surgical treatment of *Pott's disease* of the twelfth thoracic and the first lumbar vertebrae. He was afebrile. The basal metabolic rate on July 3, 1936, was minus 8 per cent with the basal pulse 86, temperature 98° F., respirations 10, blood pressure 122/80 and the weight 170 pounds.

The blood iodine on Dec. 24, 1935, was 8.2 mcg. per cent, which is low normal. The urinary iodine concentration was 7.9 mcg. (Table I, 11A) (preiodine tolerance study), which is less than that of the blood iodine. The daily urinary excretion of iodine over a period of three days (Table I, 11A), averaged 41 mcg. with the low and high limits at 26 and 68 mcg., respectively.

The urinary iodine concentration (ten days after an iodine tolerance test) was 7.2 mcg. per cent. The daily urinary excretion of iodine, over a period of eleven days (Table I, 11B), averaged 45 mcg. with the low and high limits at 17 and 86 mcg., respectively.

CASE 12.—D. C. (No. 363406) was a white male of eight years, who had *solitary bone cysts* of the upper right femur and the upper right fibula. Origin was unknown. Calcium studies of the blood and urine revealed no evidence of hyperparathyroidism. He was afebrile throughout this study. Two basal metabolic rate determinations averaged minus 7 per cent, with the basal pulse 63, temperature 98.6° F., respirations 14, blood pressure 98/60, and the weight 52 pounds.

The blood iodine on July 4, 1936, was 7.2 mcg. per cent, which is low normal. The urinary iodine concentration was 2.7 mcg. per cent (Table I, 12), which is less than that of the blood iodine. The daily urinary excretion of iodine, over a period of five days (Table I, 12), was 41 mcg. with the low and high limits 32 and 57 mcg., respectively.

CASE 13.—C. L. (No. 363527) was a white male of fifty-four years, who had a *bilateral indirect inguinal hernia*. There were no complications. The basal metabolic rate on July 10, 1936, was minus 9 per cent, with the basal pulse 65, temperature 98° F., respirations 9, blood pressure 96/66, and the weight 123 pounds.

The daily urinary excretion of iodine, over a period of eight days (Table I, 13), averaged 68 mcg. with the low and high limits at 31 and 145 mcg., respectively.

DISCUSSION

These data (Table I) reveal the great daily fluctuation in the normal urinary excretion of iodine. This physiologic variation may be due to a number of normal variants which affect iodine metabolism. Among these are the quantity[2, 21-24, 26, 28] and quality[27, 29] of the iodine intake, the activity of the thyroid gland,[21] the metabolism of the thyroid hormone in the extrathyroidal tissues, emotion[2, 21] and the excretion of iodine through other channels such as the skin and the gastrointestinal tract.[3, 12, 13, 20]

That the food intake of iodine is a factor is shown in von Fellenberg's[2] early studies. Following iodine increment to the diet, such as by the use of cod liver oil, sardines, and watercress, and by the addition of potassium iodide, there ensued an increased renal excretion of iodine. It is now known that following the administration of iodine in any form thus far investigated there is an increase in the urinary excretion of iodine. This increased excretion lasts approximately seven days after the single intravenous injection of potassium iodide.[21] However, it may last for much longer periods of time; for example after the single injection of substances such as lipiodol intraspinally.[21] Others[22-24, 28] have reported their investigations of the urinary excretion of iodine following different methods of administration of various iodine compounds. An iodine tolerance test,[25] which depends upon the estimation of the urinary iodine following single intravenous injection of a known quantity of potassium iodide, has been described. Certain organic iodine compounds owe their practical usefulness in excretion urography to their exceptionally rapid elimination in the urine.[26]

TABLE II

A COMPARISON OF THE NORMAL DAILY URINARY IODINE OF MAN IN VARIOUS GEOGRAPHIC REGIONS

REGION NO.	REGION	INVESTIGATOR	DATE OF INVESTIGATION	NUMBER OF SUBJECTS	PERIOD EACH SUBJECT WAS STUDIED	URINARY VOLUME	URINARY IODINE CONCENTRATION	RANGE IN DAILY URINARY IODINE	AVERAGE DAILY URINARY IODINE
			Nongoitrous Regions						
			YEAR		DAYS	C.C.	MCG. %	MCG.	MCG.
I	Danzig	Liek[6]	1927	5	1	–	–	200-590	343
II	Berlin, Germany	Scheringer[8]	1931	17 (nonpregnant women)	1-4	870	18.5	66-389	141
				7 men	1			103-214	173
III	Vik i Sogn, Norway	v. Fellenberg[5]	1926	8	1	–	–	94-240	146
IV	New Orleans, La.	Moore*	1934	9	10	1,555	8.3	60-270	117
V	Forte dei Marmi, Italy	v. Fellenberg[4]	1926	8	1	–	–	30-140	72
Average normal 24-hour urinary iodine (in five goiter-free regions)									165
			Goitrous Regions						
			YEAR		DAYS	C.C.	MCG. %	MCG.	MCG.
I	Effingen, Switzerland	v. Fellenberg[1]	1923	7	1	–	–	28-108	64
II	Columbus, Ohio	The Authors	1933-1936	13	3-43	1,005	5.9	7-196	51
III	Sandsvaer, Norway	Lunde[7]	1927	25	1	–	5.0	6- 83	38
IV	Lwów, Poland	Elmer and Scheps[14]	1934	2	2-4	1,200	3.0	34- 40	36
V	Pécs, Hungary	Scheffer[12]	1931-1932	5	1	1,170	2.3	21- 33	27
Average normal 24-hour urinary iodine (in five goitrous regions)									42

*We are indebted to Margaret C. Moore, who was aided by W. E. Merrill, the Department of Chemistry, Tulane University of Louisiana, New Orleans, for permission to include these unpublished data.

The excretion of iodine by the kidney not only depends upon the quantity but also upon the quality of the iodine intake. Greenbaum and Raiziss[27] showed that the elimination of iodine subsequent to the administration of calcium iodide orally; and of an addition product of calcium iodide and thiourea, orally or intravenously, is entirely different from that subsequent to the administration of sodium or potassium iodide or after some organic iodine compounds. Approximately one-half appeared in the urine and the remainder in the feces. The iodides of potassium and sodium were eliminated almost quantitatively in the urine. Elmer and Rychlik[29] report that ingestion of relatively large amounts of inorganic iodine as potassium iodide results in rapid excretion of rather large amounts of iodine in the urine, whereas ingestion of large amounts of thyroxine is followed by urinary excretion of smaller amounts of iodine. The significance of these facts remains obscure.

It is possible that a normal varying activity of the thyroid gland in the utilization of iodine is another factor which may affect the daily urinary excretion of iodine. Pathologically, the greatest increase of urinary iodine occurs in certain cases of hyperthyroidism.[21] Subsequent to total thyroidectomy in man there results a variable transient increase in the renal excretion of iodine.[30] This is followed by a lowered normal range.[30]

It has been shown by von Fellenberg,[2] and recently in this laboratory[21] that emotion is a factor in iodine metabolism. Emotional disturbances in some of our patients resulted in an increased renal excretion of iodine.

The urinary iodine, or the fecal iodine, expressed as the percentage of the total iodine excreted in both, may vary.[3, 12, 13, 20] One male, E. S. (Table I, 6),[3, 20] on a constant regimen with an average iodine intake of 468 mcg. per three-day period, excreted 64 per cent (of the total iodine excreted in the urine and feces) in the urine and 36 per cent in the feces. A normal female, G. L.,[3, 20] also on a constant regimen with an average iodine intake of 168 mcg. per three-day period, excreted 79 per cent (of the total iodine excreted in the urine and feces) in the urine and 21 per cent in the feces. These two normal subjects of twenty-eight and thirty-one years, respectively, who have been investigated for a total period of thirty days, excreted approximately twice as much iodine in the urine as in the feces.

As yet, there has been revealed no definite relationship between the urinary iodine and the amount of daily urine secretion. These data (Table I) show that an increase in the urinary iodine does not necessarily occur with an increased twenty-four-hour urinary volume neither in varying individuals nor in the same individual.

The effect of age on iodine metabolism and the urinary excretion of iodine is not definitely known. However, we observe that of the group of nine males (Table I), two males of eight years, at which age the energy metabolism is normally increased, excreted 41 micrograms (average) of urinary iodine daily over a total period of fifteen days; five males of twenty-eight to thirty-four years, at which age the energy metabolism is decreased, excreted 50 micrograms (average) of urinary iodine daily over a total period of sixty-nine days, and two males of fifty-four and fifty-nine years, when the energy metabolism is still less, ex-

creted 73 micrograms of urinary iodine daily over a total period of thirty days. It would consequently seem that there exists a relationship between the energy metabolism, which varies normally with age, and the urinary excretion of iodine. However, one should use caution in drawing such conclusions, since these are relatively few cases and other factors may be concerned.

Other normal variants related to iodine metabolism and the thyroid gland are those associated with the menstrual cycle[31] and pregnancy,[32] the metabolism of other minerals,[20] other hormones[33-35, 37] and endocrine glands,[33, 36, 55] vitamins,[38, 39, 50] exercise,[40, 41, 53] antithyreotropic protection substance,[42, 43] the absorption of iodine,[44, 51, 52] and the autonomic nervous system.[45, 49] The part which each of these factors plays in iodine metabolism is still obscure. Other investigators have studied the interrelationship of thyroid function and iodine metabolism to vitamins and other substances. These we are unable to review in the present paper.

The relation of surface area and of varying seasonal and climatic conditions to the urinary excretion of iodine has not been determined.

Even though the excretion of iodine by the kidney is normally influenced by many factors, wide variations in its excretion through this channel are not representative of ordinary conditions. This is especially true when the subject is kept on a constant regimen immediately preceding and during the period of study. Individuals maintained for days on a constant diet[3, 20] reveal that the urinary iodine excretion remains quite constant. In most normal individuals the daily renal excretion of iodine is usually limited to a definite range. This normal fluctuation is accounted for chiefly by differences in the amount of iodine in the food intake. The urinary iodine, normally, is derived to a lesser extent from the body metabolism.

Exactly what rôle the kidney plays in iodine metabolism is not known. Nor is the mechanism by which the kidney excretes iodine definitely known. It is well to remember, however, that the urinary iodine does not always represent the true picture of iodine metabolism.[20]

The form in which iodine exists in the urine has not been definitely determined. Elmer and Scheps[14] found no thyroxine present in the urine of either normal individuals or exophthalmic goiter patients. They treated the urine after the method of Leland and Foster.[46] Asimoff and Estrin[47, 48] recently found that after feeding dried thyroid gland to dogs and after intravenous injection of thyroxine, the iodine excretion in the urine is increased. This urine in no instance affected metamorphosis of the axolotl. They concluded that the iodine is excreted in the urine in simple compound form. Further investigation of these particular problems is necessary.

The significance of the fact that the concentration of iodine in the urine varies normally in different geographic regions (Table II), so that it may be lower, equal to, or greater than that of the blood iodine (Table I) is not definitely known. It would appear that the kidney has an iodine threshold.

Table II summarizes data collected by various investigators working in several widely separated geographic regions. It demonstrates that the average normal urinary excretion of iodine of man in five nongoitrous regions is ap-

proximately four times as great as that of man in five goitrous regions. A comparison of the Columbus grand average (51 micrograms) of urinary iodine excretion with those of other regions, reveals that ours is only a fraction of those of the goiter-free regions of Danzig; Berlin, Germany; Vik i Sogn, Norway; New Orleans, Louisiana; and Forte dei Marmi, Italy. It is little less than that of the slightly goitrous region of Effingen, Switzerland. However, it is greater than in the goitrous regions of Sandsvaer, Norway; Lwów, Poland, and Pées, Hungary (Table II). This difference in the urinary excretion of iodine is probably due to a difference in the absorbable iodine available for the inhabitants of these various areas.

SUMMARY

1. Iodine is a normal constituent of human urine.

2. Two hundred and twenty-seven twenty-four-hour urinary iodine determinations made on thirteen individuals without evidence of thyroid disease gave variations of from 7 to 196 micrograms per twenty-four-hour period, with averages of from 36 to 78 micrograms. The grand average was 51 micrograms per twenty-four hours.

3. The daily physiologic fluctuation in the excretion of iodine by the kidney may be great. This may be due to many normal variables.

4. Even though the excretion of iodine by the kidney is influenced normally by many factors, wide variations in its excretion through this channel are not representative of ordinary conditions. This is especially true when the subject is kept on a constant regimen immediately preceding and during the period of study. The normal daily fluctuation in urinary iodine is accounted for chiefly by differences in the amount of iodine in the food intake. The urinary iodine, normally, is derived to a lesser extent from the body metabolism.

5. The relation of age to iodine metabolism is, as yet, unknown. However, we observe that of a group of nine males, two of eight years excreted 41 micrograms (average) of urinary iodine daily over a period of fifteen days; five of twenty-eight to thirty-four years, excreted 50 micrograms (average) of urinary iodine daily over a period of sixty-nine days; and two of fifty-four and fifty-nine years, excreted 73 micrograms (average) of urinary iodine daily over a period of thirty days.

6. The average iodine excretion of normal individuals in central Ohio is less than that of normal man of the five goiter-free regions of Danzig; Berlin, Germany; Vik i Sogn, Norway; New Orleans, Louisiana; and Forte dei Marmi, Italy. It is little less than that of the slightly goitrous region of Effingen, Switzerland. But it is greater than in the goitrous regions of Sandsvaer, Norway; Lwów, Poland, and Pées, Hungary.

7. The normal urinary excretion of iodine of man in five nongoitrous regions is approximately four times as great as that of man in five goitrous regions. This difference in urinary excretion of iodine is probably due to a difference in the absorbable iodine available for the inhabitants of these various regions.

8. A clinical interpretation of a determination of the urinary iodine requires a thorough understanding of the physiologic variables which influence iodine metabolism. Further investigation of these variables is needed.

REFERENCES

1. v. Fellenberg, Th.: Untersuchungen über das Vorkommen von Jod in der Natur. VI. Mitteilung: Über den Zusammenhang zwischen der Häufigkeit des Auftretens von Kropf und dem Jodgehalt der Umwelt, Biochem. Ztschr. **152**: 141, 1924.
2. v. Fellenberg, Th.: Das Vorkommen, der Kreislauf und der Stoffwechsel des Jods, Ergebn. d. Physiol. **25**: 176, 1926.
3. Cole, V. V., and Curtis, G. M.: Human Iodine Balance, J. Nutrition **10**: 493, 1935.
4. v. Fellenberg, Th.: Untersuchungen über den Jodstoffwechsel. II. 1. Über den normalen Jodstoffwechsel, Biochem. Ztschr. **174**: 341, 1926.
5. v. Fellenberg, Th.: Untersuchungen über den Jodstoffwechsel. III. Biochem. Ztschr. **184**: 85, 1927.
6. Liek, E.: Ist die Jodmangeltheorie des Kropfes richtig? München. med. Wchnschr. **74**: 1786, 1927.
7. Lunde, G.: Untersuchungen über den Jodstoffwechsel. I. Mitteilung: Über die Jodausscheidung durch den Harn von Bewohnern eines norwegischen Kropfgebietes, Biochem. Ztschr. **193**: 94, 1928.
8. Scheringer, W.: Die Jodausscheidung im Harn der Schwangeren unter physiologischen und experimentellen Bedingungen, Arch. f. Gynäk. **145**: 701, 1931.
9. McCarrison, R., Sankaran, G., and Madhava, K.: Urinary Excretion of Iodine by Goitrous and Non-Goitrous Persons in Gilgit, Indian J. M. Research **18**: 1335, 1931.
10. McCarrison, R., and Sankaran, G.: Urinary Excretion of Iodine by Goitrous and Non-Goitrous Persons in Gilgit, Indian J. M. Research **19**: 67, 1931.
11. Scheffer, L.: Jodgehalt der Nährung und Kropfhäufigkeit, Biochem. Ztschr. **247**: 418, 1932.
12. Scheffer, L.: Über die Jodbilanz normaler Menschen, Biochem. Ztschr. **259**: 11, 1933.
13. Scheffer, L.: Jodstoffwechsel bei Schilddrüsenkranken, Klin. Wchnschr. **12**: 1285, 1933.
14. Elmer, A. W., and Scheps, M.: La thyroxine est-elle éliminée par les reins chez l'Homme normal et chez les basedowiens? Compt. rend. Soc. de Biol. **115**: 968, 1934.
15. Cole, V. V., Curtis, G. M., and Bone, M. L.: The Iodine Content of Hospital Foods, J. Am. Dietet. A. **10**: 200, 1934.
16. Phillips, F. J., and Curtis, G. M.: Blood Iodine Studies. IV. The Clinical Determination of Iodine in Blood, Urine, and Feces, Am. J. Clin. Path. **4**: 346, 1934.
17. Matthews, N. L.: Personal communication.
18. Leipert, Th.: Die Bestimmung kleinster Jodmengen in organischem Material, Biochem. Ztschr. **261**: 436, 1933.
19. Leipert, Th.: Zur Kenntnis des physiologischen Blutjodspiegels, Biochem. Ztschr. **270**: 448, 1934.
20. Puppel, I. D., and Curtis, G. M.: Calcium and Iodine Metabolism in Thyroid Disease, Arch. Int. Med. **58**: 957, 1936.
21. Curtis, G. M.: Unpublished data.
22. Kendall, E. C.: The Physiologic Action of Thyroxin, Endocrinology **3**: 156, 1919.
23. Cattell, R. B.: The Elimination of Iodine in the Urine in Normal Persons and in Exophthalmic Goiter, Boston M. & S. J. **195**: 69, 1926.
24. Krayer, O.: Über Verteilung und Ausscheidung des Jodes nach Zufuhr von Schilddrüsenstoffen, Arch. f. exper. Path. u. Pharmakol. **128**: 116, 1928.
25. Elmer, A. W.: Iodine Tolerance Test for Thyroid Insufficiency, Endocrinology **18**: 487, 1934.
26. Elsom, K. A., Bott, P. A., and Landis, E. M.: Simultaneous Excretion of Creatinine and Certain Organic Iodine Compounds, Proc. Soc. Exper. Biol. & Med. **32**: 77, 1934.
27. Greenbaum, E. R., and Raiziss, G. W.: The Elimination of Iodine After Oral or Intravenous Administration of Various Iodine Compounds in Single Massive Doses, J. Pharmacol. & Exper. Therap. **30**: 407, 1926-27.
28. Barnes, B. O.: Iodine Excretion in Experimental Hyperthyroidism, Am. J. Physiol. **103**: 699, 1934.
29. Elmer, A. W., and Rychlik, W.: Recherches comparatives sur l'excrétion d'iode par l'urine, après ingestion d'iode minéral, de thyroxine et de diiodotyrosine, Comp. rend. Soc. de Biol. **115**: 1719, 1934.
30. Curtis, G. M., and Barron, L. E.: The Urinary Loss of Iodine Following Total Thyroidectomy, Surgery **1**: 92, 1937.
31. Cole, V. V., and Curtis, G. M.: Cyclic Variations in Urinary Excretion of Iodine in Women, Proc. Soc. Exper. Biol. & Med. **31**: 29, 1933.
32. Enright, L., Cole, V. V., and Hitchcock, F. A.: Basal Metabolism and Iodine Excretion During Pregnancy, Am. J. Physiol. **113**: 221, 1935.
33. Houssay, B. A.: The Hypophysis and Metabolism, New England J. Med. **214**: 961, 1936.

34. Schockaert, J. A., and Foster, G. L.: Influence of Anterior Pituitary Substances on the Total Iodine Content of the Thyroid Gland in the Young Duck, J. Biol. Chem. **95**: 89, 1932.
35. Closs, K., Loeb, L., and MacKay, E. M.: The Effect of an Acid Extract of the Anterior Pituitary on the Blood and Thyroid Gland, J. Biol. Chem. **96**: 585, 1932.
36. Marine, D.: Remarks on the Pathogenesis of Graves' Disease, Am. J. M. Sc. **180**: 767, 1930.
37. Stimmel, B. F., McCullagh, D. R., and Picha, V.: The Thyrotropic Hormone of the Pituitary Gland and Iodine Metabolism, J. Pharmacol. & Exper. Therap. **57**: 49, 1936.
38. Abelin, I.: Über den Antagonismus A-Vitamin: Thyroxin, Ztschr. f. Vitaminforsch. **4**: 120, 1935.
39. Löhr, H.: Beiträge zur Kenntnis des Jodstoffwechsels, II. Mitteilung: Die Beeinflussung des Blutjodspiegels durch Ascorbinsäure, Arch. f. exper. Path. u. Pharmakol. **180**: 344, 1936.
40. McCullagh, D. R.: Studies in Blood Iodine by the Use of a New Chemical Method, Cleveland Clinic Quart. **2**: 15, 1935.
41. Löhr, H.: Über Jodaufnahme aus der Umwelt, Der Chirurg. **8**: 107, 1936.
42. Collip, J. B.: Recent Studies on Anti-Hormones, Ann. Int. Med. **9**: 150, 1935.
43. Loeser, A.: Untersuchungen über den Wirkungsmechanismus der antithyreotropen Schutzsubstanz des Blutes, Arch. f. exper. Path. u. Pharmakol. **181**: 173, 1936.
44. Heath, C. W., and Fullerton, H. W.: The Rate of Absorption of Iodide and Glycine From the Gastrointestinal Tract in Normal and Disease Conditions, J. Clin. Investigation **14**: 475, 1935.
45. Klein, J.: The Correlation of Mineral Metabolism and the Vegetative Nervous System in Thyroid Disease, Ann. Int. Med. **8**: 798, 1935.
46. Leland, J. P., and Foster, G. L.: A Method for the Determination of Thyroxine in the Thyroid, J. Biol. Chem. **95**: 165, 1932.
47. Asimoff, G., and Estrin, E.: Über das Schicksal des Schilddrüsenhormons im Organismus hyperthyreoidisierter Tiere. II. Mitteilung: Der Charakter der Ausscheidung des Schilddrüsenhormons nach peroraler Einführung getrockneter Schilddrüse beim Hunde, Ztschr. f. d. ges. exper. Med. **76**: 380, 1931.
48. Asimoff, G., and Estrin, E.: Über das Schicksal des Schilddrüsenhormons im Organismus hyperthyreoidisierter Tiere. III. Mitteilung: Der Charakter der Ausscheidung des Schilddrüsenhormons nach Einführung von Thyroxine per os, Ztschr. f. d. ges. exper. Med. **76**: 399, 1931.
49. Friedgood, H. B.: The Relation of the Sympathetic Nervous System and Generalized Lymphoid Hyperplasia to the Pathogenesis of Exophthalmic Goiter and Chronic Lymphoid Leukemia, Am. J. M. Sc. **183**: 841, 1932.
50. Rabinowitch, I.: The Effects of Iodine Treatment, With and Without Vitamins, on the Basal Metabolic Rate in Exophthalmic Goiter, J. Canad. M. A. **21**: 156, 1929.
51. Salazar, L.: Influenza della dose, del catione e dell' alimentazione sull' assorbimento degli ioduri salini, Clin. med. ital. **58**: 37, 1927.
52. Blumgart, H., Gilligan, D., and Swartz, J.: The Elimination of Ethyl Iodide After Inhalation and Its Relation to Therapeutic Administration, J. Clin. Investigation **9**: 635, 1930-31.
53. Herxheimer, H., Mislowitzer, E., and Stanoyévitch, L.: Sur la quantité d'iode présente dans le sang pendant le travail musculaire intensif, Compt. rend. Soc. de Biol. **115**: 886, 1934.
54. Szász, E.: Über Jodausscheidung gesunder und hyperthyreoter Menschen im Höhenklima, Med. Klin. **29**: 1584, 1933.
55. Black, E., Hupper, M., and Rogers, J.: The Effects of Adrenal Feeding Upon the Iodine Content of the Thyroid Gland, Am. J. Physiol. **59**: 222, 1922.

ANAPHYLACTOID REACTIONS TO COLLOIDAL SULPHUR IN GUINEA PIGS*

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IN THE course of a series of experiments on pollen anaphylaxis in guinea pigs in which a colloidal sulphur preparation was used in combination with pollen, certain reactions were obtained which were of considerable interest in view of the widespread use of sulphur in the treatment of various clinical syndromes. The pharmacologic action of sulphur is dependent apparently on the formation of sulphur compounds with hydrogen, the halogens, and organic matter. The toxicity of colloidal sulphur varies from 7 mg. to 315 mg. per kilo body weight when administered intravenously in rabbits (Messini[1]). The dispersion of the particles and the rate of injection have also been found to influence the toxic level.

Lawson and others[2] noted that sulphur, when ingested by guinea pigs and rabbits, produces a necrosis of the stomach, and a severe nephritis; use of a 1 per cent colloidal sulphur intracardially in rabbits and guinea pigs resulted in several fatal reactions attributable to emboli.

Wheeldon and Main[3] found that the toxic dose of aqueous sulphur diasporal (S-D) injected intraperitoneally in guinea pigs ranged from 30 to 40 mg. per kilo body weight; that of the oil preparation was 220 mg. per kilo body weight. In rabbits, the intravenous toxic dose varied from 6.6 mg. to 15 mg. per kilo body weight, depending upon the speed of injection. They concluded that this particular preparation was not harmful in clinical doses.

Attention is directed in the following experiments to the anaphylactoid reactions which appear when colloidal sulphur is administered intravenously in guinea pigs.

MATERIALS AND METHODS

The guinea pigs were of both sexes weighing between 256 and 540 gm., averaging 445 gm. in the treated groups (I and III), and about 341 gm. in the untreated group (II).

I. Consisted of 3 subdivisions as follows:

A. Sensitized with 3 subcutaneous injections, 0.4 c.c. each of equal parts of colloidal sulphur and pollen

Bo. Three subcutaneous injections 0.4 c.c. sulphur at four-day intervals and three weeks later 3 subcutaneous injections 0.4 c.c. sulphur at four-day intervals.

C. Three injections 0.4 c.c. sulphur at four-day intervals.

II. Consisted of animals which had not received any previous injections.

D. Aqueous sulphur preparation
B. Sulphur in oil preparation
} amounts as noted in table.

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Received for publication, December 9, 1936.

III. Consisted of animals which had received previous control injections, *not sulphur.*

Ao. Given horse serum 0.4 c.c. 3 injections at four-day intervals.

B. Three injections 0.4 c.c. pollen at four-day intervals.

The compounds* used in these experiments were:

a. An aqueous preparation of highly dispersed colloidal sulphur suspended in 3 per cent dextrose, with a pH of 7.8 to 8.3. The ratio of colloidal sulphur to the total sulphur content is 1: 1.46—the sulphur apart from the colloidal form, being polysulphides necessary for the stability of the preparation. The size of the sulphur particles ranges from 3 to 5 millimicra. Each 2 c.c. contains 10 mg. of colloidal sulphur.

b. A neutral colloid suspension of sulphur compound (magnesium salt of sulphurated arachic acid) suspended in acid free olive oil; size of the particles about 3 millimicra, each 2 c.c. representing 25 mg. of sulphur.

In the treated group, the shock experiments were done approximately five weeks after the last sensitizing dose. In the untreated group, shocking was performed two days after their arrival in the laboratory. For this, the jugular vein was exposed and varying amounts of the preparation injected. In several cases, the sulphur was administered intraperitoneally. Each animal was observed for at least two hours and all reactions noted. In Animals 80, 91, 93, 95, and 96, histologic examinations of the lungs were made on preparations stained with hematoxylin and eosin. In Animals 89, 90, 94, and 100, lung sections were also stained with osmic acid. Sulphur precipitins were investigated in the prepared group after the method of Hektoen, using blood serum diluted 1:3 with normal saline; no precipitins could be demonstrated.

RESULTS

The predominant manifestations were sudden collapse, weakness, convulsions, and terminal dyspnea with respiratory death. The reactions began within ten to twenty seconds, death occurring in ten to one hundred eighty seconds in those animals receiving a fatal dose. Toxic effects were produced with all intravenous doses including the preparation diluted 1:100 (Animal 69). Intraperitoneal injections proved ineffectual in doses 3 times the intravenous quantity. Wells[4] has estimated that intraperitoneal injections require doses 50 times the intravenous amount to secure equally severe reactions, much depending on the solubility of the preparation in the peritoneal fluid. It was observed that the faster the speed of injection, the more rapid was the effect.

In Group I the A.L.D. (average lethal dose) of the aqueous preparation =0.4 c.c., or 4.9 mg. per kilo body weight

In Group II the A.L.D. of aqueous preparation = 0.2 c.c., or 3.5 mg. per kilo body weight

The average lethal dose of the sulphur in oil preparation was 45 mg. per kilo body weight. The anaphylactoid response was less marked in Group I than in Group II. This would suggest that preparatory injections of the drug tend to reduce the incidence of the reaction (see Table I). All of the above doses are far in excess of the amounts recommended for clinical use.

*Kindly furnished by the Doak Company, Cleveland, Ohio. Information furnished by the Doak Company.

Postmortem Findings.—The lungs were found to be collapsed; there was evidence of mild congestion on the cut surface. The heart showed no gross changes. Histologically, the pulmonary arteries were well collapsed and the bronchi moderately contracted (not beyond normal limits). In 2 sections, slight perivascular edema was noted. There was the usual interstitial round cell infiltration and the presence of a moderate number of eosinophiles. In sections from Animals 95 and 96, a rather definite "hyaline" fusion of deeply staining red blood cells was noted. This could not be attributed to delayed fixation of the specimens and was considered as possible evidence of early thrombus formation. Both of these animals received the oil preparation intravenously. This was not observed in any of the other sections.

TABLE I*

	ANIMAL	WEIGHT	AMOUNT	PREP'N	RESPONSE	AVERAGE LETHAL DOSE PER KILO BODY WEIGHT
Group I						
A	81	288 gm.	0.4 c.c.	Aq.	4+	6.6 mg.
	78	256	0.8 1:10	Aq.	2+	
	91	360	0.4	Aq.	4+	5.5 mg.
	74	500	0.2	Aq.	2+	
	96	310	0.5	Aq.	4+	8.0 mg.
Bo	82	460	0.4 c.c. 1:10	Aq.	0-1+	
	62	490	0.4	Aq.	3+	
	54	528	0.4	Aq.	4+	1.8 mg.
	75	376	0.4 1:10	Aq.	0	
			0.4 undil.	Aq.	4+	5.3 mg.
C	70	534	0.25	Aq.	2+	
	51	520	0.40 c.c.	Aq.	4+	3.8 mg.
	52	534	0.4	Aq.	3-4+	
Group II						
D	94	294	1.0 c.c. intrap.	Aq.	0	
			0.35 intrav.	Aq.	4+	2.4 mg.
	92	365	0.1 c.c.	Aq.	4+	1.3 mg.
	91	320	0.2 c.c.	Aq.	4+	3.1 mg.
	93	390	0.2 c.c.	Aq.	4+	2.5 mg.
	89	388	0.1 c.c.	Aq.	2+	
			0.4 c.c.	Aq.	4+	5.1 mg.
	90	298	0.05	Aq.	2+	
			0.4 c.c.	Aq.	4+	6.7 mg.
B	95	304	1.0 c.c.	Ol.	4+	41.0 mg.
	97	374	0.5 c.c.	Ol.	1-2+	
	98	406	2.0 c.c. intrap.	Ol.	0-1+	
	96	331	0.8 c.c.	Ol.	4+	32.0 mg.
	99	287	2.3 c.c. intrap.	Ol.	0	
	100	343	0.3 c.c.	Ol.	1+	
			1.7 c.c.	Ol.	4+	62.0 mg.
Group III						
Ao	60	520	0.2 c.c.	Aq.	3+	
	56	540	0.35 c.c.	Aq.	4+	6.4 mg.
	69	536	1.0 c.c. 1:100	Aq.	0-1+	
B	86	400	0.8 c.c. 1:100	Aq.	2+	
	76	498	0.3 c.c.	Aq.	3+	
	89	390	0.15 c.c.	Aq.	3+	1.9 mg.

*Anaphylactoid Response =
++++ Fatal—convulsions.
+++ Collapse—marked dyspnea with recovery.
++ Weakness, flaccidity, mild dyspnea, cough.
+ Cough, scratching of nose, nervousness, sneezing.
Aq., sulphur in aqueous sol.
Ol., sulphur in oil.

COMMENT

These reactions, which resemble acute anaphylactic shock, differ from it in several important respects.

Equally severe reactions were obtained in "normal" as well as "prepared" animals. Nonfatal shocks did not desensitize the subjects. The lungs were collapsed, and microscopically they did not exhibit the usual anaphylactic bronchospasm and marked contraction of the larger pulmonary vessels. Reactions with the virgin guinea pig uterine strip were not investigated. Several in vitro experiments were conducted in an effort to reveal the nature of this phenomenon. The admixture of 1 drop of the aqueous preparation to 1 drop of guinea pig blood resulted in an immediate change in color, which gradually developed into a deep brown. This was not observed with the oil preparation. Similar color changes were noted when oxalated blood and blood plasma (guinea pig) were used. Mixing colloidal sulphur and guinea pig blood serum produced no observable change. Microscopically, the aqueous sulphur preparation caused a mild destruction of the red blood cells, noticeable within forty minutes and slightly advanced in one and one-half hours. While capillary thrombosis and embolism have been demonstrated in other experimental anaphylactoid reactions, in only 2 of the sections was any evidence of embolism noted. In each case, considerable doubt as to the significance of this finding was aroused because of the lack of additional pathologic criteria. That "fat embolism" may be a cause of death when oil preparations are used intravenously is, of course, very likely. However, only in Animal 100 was this found, and it received a rather large amount of the oily preparation. The actual pathologic findings do not explain the sudden and marked symptoms which the animals exhibited. The changes were too rapid to be attributable to the action of the sulphur itself. Other explanations have been sought in the realm of colloidal chemistry. Hanzlik and Karsner[5] could not show that the colloids which they used, such as agar, gelatin, and starch, altered the ferment-antiferment balance, the coagulation of blood, or affect nerve centers, ganglia, or myoneural junctions. As regards the latter, Steiner[6] has demonstrated pseudoanaphylaxis with benzene in guinea pigs whose brain and cord had been pithed. Toxic action upon the capillaries and increased capillary permeability have been advanced as another explanation. This theory finds an experimental basis in the investigations of De Eds and Mitchell[7] and Hanzlik and others,[8] who believe that resultant changes in cell surfaces disturb the normal equilibrium existing between tissues and body fluids, thus causing alterations in their functional state or activity. The effects are apparently due to some vague physicochemical change induced by the injected compounds. In view of the general characteristics of colloids, their relationship to anaphylactoid reactions remains yet to be conclusively demonstrated. According to Kolmer,[9] colloids possessing small osmotic pressures themselves, exert slight influence upon the osmotic pressure, freezing point, and boiling point of fluids. Zunz and La Barre, however, studying the physicochemical changes in serum during anaphylactoid shock in guinea pigs, found a lowering of surface tension, alteration of the pH and index of refraction, and a definite increase in the A/G ratio. Hanzlik and others[11] have also noted changes in the A/G ratio of serum and plasma,

sedimentation rate, and red cell fragility. In the dog, increased lactic acid and ammonia have been found; also a darkening of the blood, with agglutination and hemolysis of the red cells. The work of Hanzlik and his associates represents a definite advance in clarifying the mechanism of the anaphylactoid reaction. Of interest are several articles[12, 13] in the French literature, which report protection against such reactions by the use of fluorescent dyes and magnesium thiosulphate. Some light may perhaps be thrown on the whole subject by this approach.

Clinically, this problem is of great significance. Waldbott,[14] who has written extensively on these reactions, reported 3 cases of possible anaphylactoid shock, one after the injection of 0.6 gm. of neoarsphenamine and two following the use of highly concentrated glucose. Brines[15] has described similar reactions after blood transfusions. Many substances, then, are seen to produce this alarming phenomenon. Though an injected compound may be of apparent clinical efficacy, the inability accurately to predict its possible untoward effects becomes a serious disadvantage. Until the nature of anaphylactoid phenomena is more clearly elucidated, it would seem advisable to use only those new therapeutic agents whose value in any given condition is such that with a constant alertness for this complication, their continued use is warranted.

SUMMARY

1. Colloidal preparations of sulphur when administered intravenously in guinea pigs produce anaphylactoid reactions.

2. Previous injections of sulphur apparently diminish the incidence and severity of the anaphylactoid response to sulphur.

3. Differences in the aqueous and oleous preparations are pointed out.

4. The lethal dose in guinea pigs is far in excess of the amounts recommended for clinical use.

5. The anaphylactoid reaction is discussed briefly.

REFERENCES

1. Messini, M.: Dispersitätsgrad und pharmakologische Wirkung des Kolloiden Schwefels, Arch. f. Exper. Path. u. Pharmakol. **127**: 366, 1928.
2. Lawson, G. B., Redfield, K. T., and Boyce, O. D.: Toxic Effects of Sulphur in Guinea Pigs and Rabbits, J. Lab. & Clin. Med. **20**: 169, 1934.
3. Wheeldon, T. F., and Main, R. J.: Use of Colloidal Sulfur in Treatment of Arthritis, J. Bone & Joint Surg. **15**: 94, 1933.
4. Wells, H. G.: Chemical Aspects of Immunity (American Chemical Society Monograph Series), New York, 1925.
5. Hanzlik, P. J., and Karsner, H.: Anaphylactoid Phenomena From Intravenous Administration of Various Colloids, Arsenicals, and Other Agents, J. Pharmacol. & Exper. Therap. **14**: 379, 1920 (Excellent bibliography).
6. Steiner, P. E.: Fatal Pseudo-Anaphylaxis by Intramuscular Injection of Benzene and Related Substances, J. Immunol. **27**: 525, 1934.
7. De Eds, F., and Mitchell, V.: Thrombocyte and Erythrocyte Changes Produced by Agents Causing Anaphylactoid Reactions, J. Pharmacol. & Exper. Therap. **28**: 433, 1926.
8. Hanzlik, P. J., Empey, L. W., De Eds, F., and Farr, W. H.: Hemoclastic Changes In Vitro From Agents Causing Anaphylactoid Reactions, J. Pharmacol. & Exper. Therap. **32**: 273, 1928.
9. Kolmer, J. A.: Infection, Immunity and Biologic Therapy, Philadelphia, 1924, W. B. Saunders Co., p. 576.

10. Zunz, LaBarre: Trans. L'Inst. Therap. Bruxelles **16**: 1922. (Quoted by Hanzlik.[8])
11. Hanzlik, P. J., De Eds, F., and Tainter, M. L.: Blood and Symptomatic Changes Following Intravenous Administration of Variety of Agents and Solutions, Arch. Int. Med. **36**: 447, 1925.
12. Girard, P., and Peyre, E.: Physiologic Consequences of Modification of Colloidal State of Plasma by Certain Fluorescent Dyes; Prevention of Anaphylactic Sensitization, Compt. rend. Soc. de biol. **95**: 181, 1925.
13. Lumiere, A., and Malespine, Mme.: Hyposulfite de Magnésium et choc Anaphylactique Compt. rend. Soc. de Biol. **100**: 351, 1929.
14. Waldbott, G. L.: Allergic Shock From Substances Other Than Pollen and Serum, Ann. Int. Med. **7**: 1308, 1934.
15. Brines, O. A.: Fatal Post-transfusion Reactions, J. A. M. A. **94**: 1114, 1116, 1930.

VARIATIONS IN THE TOXICITY OF MORPHINE SULPHATE*

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IN THE recent literature, we find an accumulation of clinical observations and experimental evidence of the fact that the physiologic state of the body varies continually (Petersen[1]). This led us to undertake certain experiments which may explain the variations in toxicity of known drugs that give difficulties in estimation of their therapeutic and lethal doses.

The experiments were carried out in the following manner: A number of young mice, approximately of the same age, of 17 to 21 gm. of weight were put on the diet of "Special Meal" (of Old Trusty Dog Food Co.). Free use of water was permitted. This diet produced in urine a pH of 6.0.

Each day (except Saturdays and Sundays) for seven weeks, in a group of ten mice was evoked the "Straub sign" (a peculiar curling and stiffening of the tail due to injection of a small amount of morphine). It has been produced by injecting subcutaneously into the back of a mouse morphine sulphate in dose of 0.4 mg. in 0.2 c.c. of water. This dose, according to Herrmann,[2] should evoke the Straub sign in about twelve minutes, with the duration of maximal reaction for about 2¾ hours and the total duration of the reaction for four hours. The animals were observed from the time of the morphine injection until the end of the reaction and the Straub sign recorded, for the first 1½ hours, every fifteen minutes, and, later, every thirty minutes, until the reaction disappeared in all mice. The results are presented on Graph 1.

Each vertical shaded column represents a summary of an experiment (10 mice) performed on a certain day, the dates being given at the bottom. The shaded area designates the positive Straub sign. Figures at the left present the time interval at which the Straub sign has been recorded, beginning from the moment of morphine injection.

On the left side of the graph are given separately the findings of the experiments, performed on the first day with explanations in detail. The first

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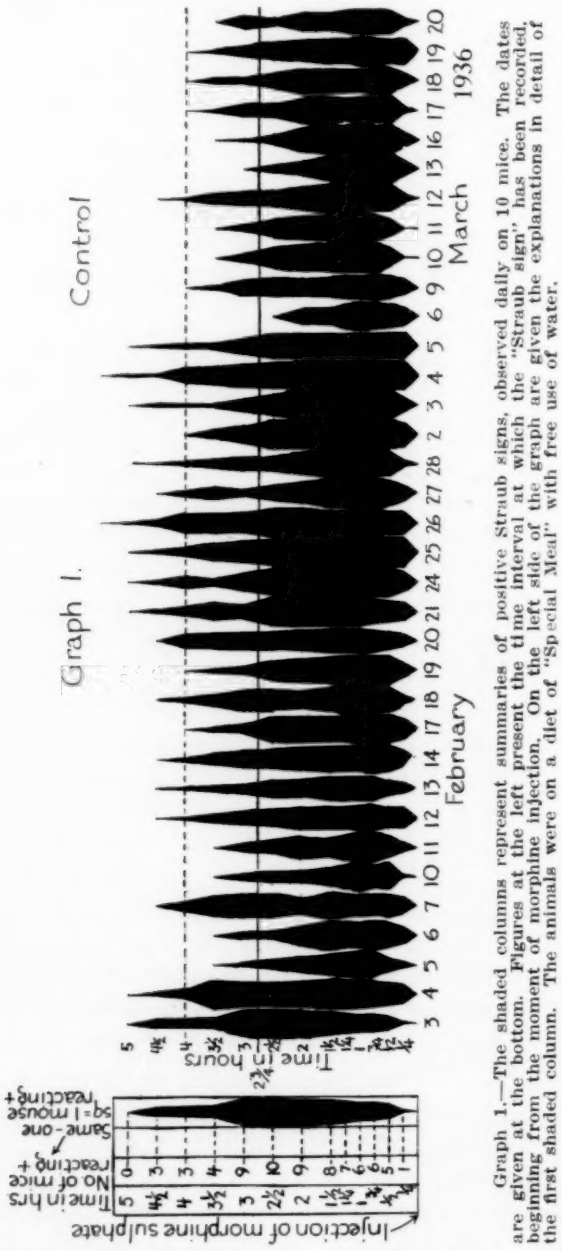
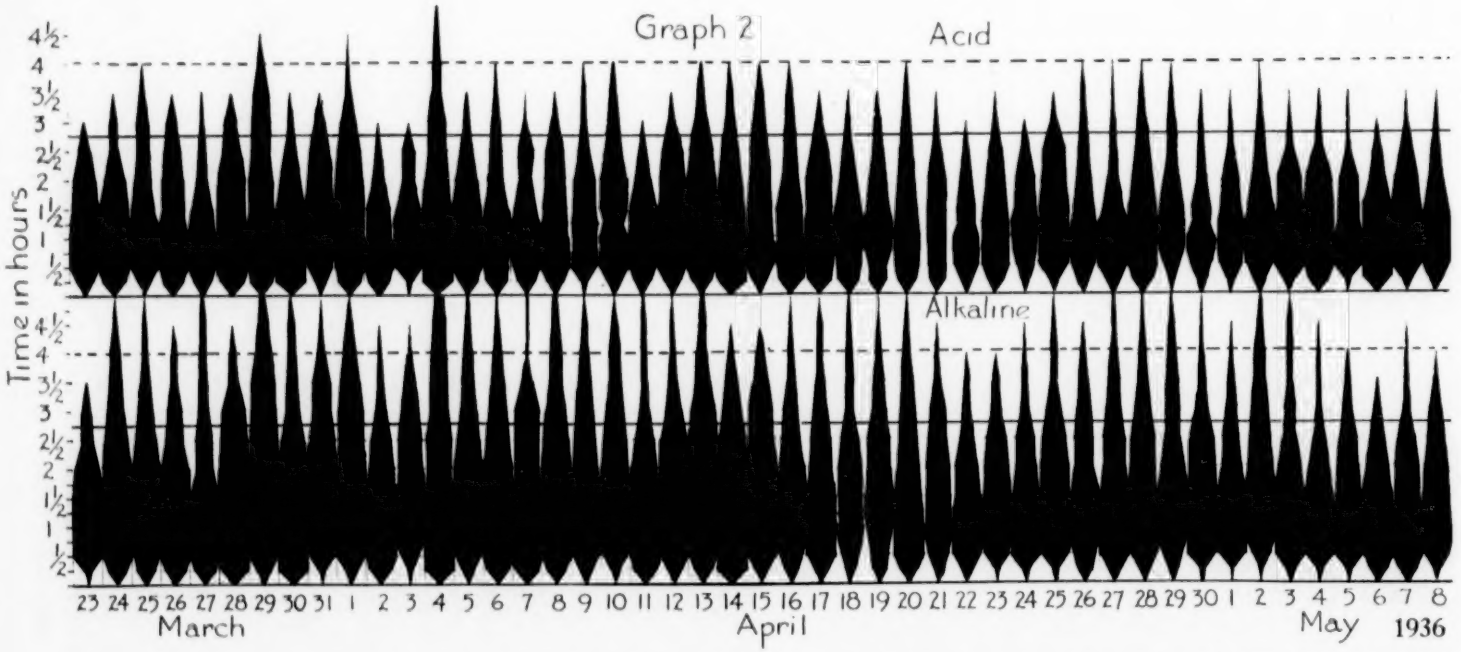
Received for publication, December 29, 1936.

column shows the time interval of recording the Straub sign in animals under observation from the moment of injection of morphine. The second column gives number of mice reacting positively at the time of recording. The last



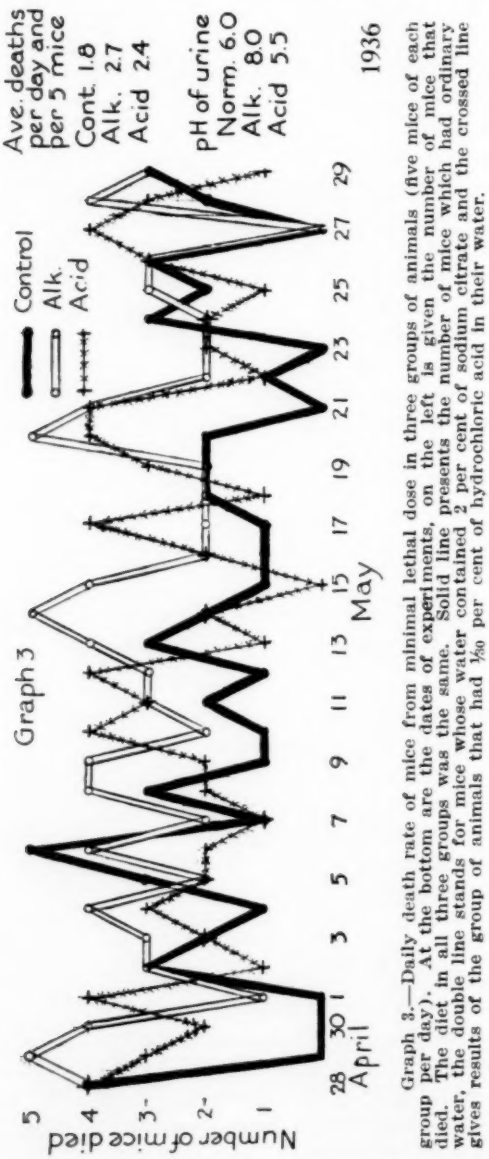
Graph 1.—The shaded columns represent summaries of positive Straub signs, observed daily on 10 mice. The dates are given at the bottom. Figures at the left present the time interval at which the "Straub sign" has been recorded, beginning from the moment of morphine injection. On the left side of the graph are given the explanations in detail of the first shaded column. The animals were on a diet of "Special Meal" with free use of water.

column presents these numbers graphically, as it is given in Graphs 1 and 2. The horizontal heavy, solid line (at 2¾ hours) over the entire graph designates the time of duration of the maximal reaction and the dotted line (at four hours)—the total duration of it, both according to Herrmann.[2]



Graph 2.—Same as on Graph 1. Here are presented the findings in the animals of two groups which were on the same diet, except that the first group (upper row) had in their water 1/50 per cent of hydrochloric acid and in the second group (lower row) the mice had water which contained 2 per cent of sodium citrate.

The study of the graph reveals great variability in the degree of reactions in the animals to the morphine injections. On some days the reaction still is positive in some animals after five hours while on other days it ceases in 2½ hours. On certain days the majority of animals react positively for four hours;



Graph 3.—Daily death rate of mice from minimal lethal dose in three groups of animals (five mice of each group per day). At the bottom are the dates of experiments, on the left is given the number of mice that died. The diet in all three groups was the same. Solid line presents the number of mice which had ordinary water, the double line stands for mice whose water contained 2 per cent of sodium citrate and the crossed line gives results of the group of animals that had 1/50 per cent of hydrochloric acid in their water.

and, on other, two hours is the limit. On the whole our average figures will correspond with such of Herrmann's, but, studied separately by days, they show very pronounced variations.

Previously, we have (Nedzel[3, 4]) studied the influence of diets upon the toxicity of some drugs. So we introduced this factor in our experiments in the following manner: A number of mice, kept on the same standard (for our

animals) diet, were divided in two groups. To the water of one group we added 2 per cent of sodium citrate and to the water of the other group $\frac{1}{30}$ per cent of hydrochloric acid. This raised the pH of the urine of the first group up to 8.0 and lowered it in the other to 5.5. Daily, for forty-seven consecutive days, ten mice of each group were treated as in the experiments described above. The results are recorded on Graph 2. The upper part of the graph represents the findings in the group of mice which were on water with hydrochloric acid and whose urine reacted acid, while the lower part—those whose urine was definitely alkaline.

The graph shows the same great variability in the Straub sign of the animals in both groups. Each day the Straub sign in mice varied, being either prolonged or shortened, more pronounced or slighter, affecting all or few mice at the time. If we compare the findings in both groups, in total, then we see a definite general difference in reactions of mice in acid and alkaline groups. The upper group (acid) reacts less, the duration of the Straub sign being shorter. In the second group (alkaline) this sign was definitely prolonged. In all these experiments there were observed very few deaths which could be attributed to the toxic effect of morphine.

We next studied the differences in reaction of animals to the minimal lethal dose of morphine, taking as such 0.25 gm. per kilo of animals, which makes 5 mg. per mouse of 20 gm. of weight.

In this series of experiments we had three groups of mice, all kept on the same standard diet. The first group (used as control) was on plain water, the second group had 2 per cent sodium citrate, and the third one, $\frac{1}{30}$ per cent of hydrochloric acid in their water. Each consecutive day five animals from each group were used. Every animal was weighed separately and given the dose corresponding to 5 mg. per 20 gm. of weight. The animals were observed either until their death or recovery and results recorded. The findings are presented on Graph 3.

The abscissa presents the dates on which the experiments were performed, and the ordinates, the number of mice which died on that particular date. Here the results are more striking than in experiments with the Straub sign. Though the average death rate per day and per five mice was 1.8 for the control group, 2.7 for the alkaline group, and for the acid group 2.4, nevertheless the daily variations were great. There were days when even the normal group, which has the lowest death rate, either would have no deaths or 100 per cent death rate. For alkaline and acid groups we find days with 20 per cent deaths and days with 100 per cent. There was a day when the normal group showed a higher mortality than the alkaline group, though the latter one has a higher average death rate. There are also reversed findings for normal and acid groups, as well as for acid and alkaline groups.

CONCLUSIONS

1. Daily observations of the Straub sign in mice show very noticeable variations in animals on standard diet as well as on addition of alkali or acid. Nevertheless it is clear that the mice on alkaline diet are more responsive to the Straub sign than the animals on acid diet.

2. The minimal lethal dose of morphine sulphate in mice varies considerably by day. While the diets, which change the pH of the urine, produce striking variations in the minimal lethal dose of morphine sulphate, the change in the case of both acid and alkaline diets appears to be in the same direction.

It is my pleasant duty to express sincerest gratitude to Dr. Bernard Fantus for his suggestions and criticisms, which proved of inestimable value.

REFERENCES

1. Petersen, Wm. F.: The Patient and the Weather, Vol. I-IV, Ann Arbor, Mich., 1934-36, Edwards Brothers.
2. Herrmann, O.: Biologische Nachweise des Morphins, Biochem. Ztschr. **39**: 216, 1912.
3. Nedzel, A. J.: Cocaine Poisoning as Influenced by Diet, J. LAB. & CLIN. MED. **19**: 874, 1934.
4. Nedzel, A. J.: Influence of Diet Upon the Toxicity of Ethylhydrocupreine Hydrochloride, J. LAB. & CLIN. MED. **20**: 944, 1935.

LABORATORY METHODS

THE CHEMICAL DIAGNOSIS OF PREGNANCY BY DETECTION OF ESTRIN IN URINE*

II. A Note on the Hydrolysis of Estrin Esters

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WE WISH to report briefly at this time experimental data which we have obtained concerning the optimum acidity for the hydrolysis of estrin esters in our chemical procedure for pregnancy diagnosis.[1]

It may be well for the sake of clarity to summarize the principles of this procedure at this point. The estrin esters of the twenty-four-hour urine are hydrolyzed by boiling and concentrating the urine which previously has been adjusted to pH 4 with HCl. The free estrin and free phenols are then extracted with ether. Aromatic hydroxy acids and pyrocatechin are removed from the ether by sodium carbonate washing. The ether is distilled off and the residue is freed of volatile phenols by distillation with water. The water is removed, the hormone residue is coupled with diazotized para-nitroaniline, and the amount of azo dye formed estimated against a standard solution of ferric chloride. The result is expressed as the "ferric chloride number" (FN), which is the colorimeter reading of the ferric chloride solution against the color test placed at 10 mm. A ferric chloride number over 25 indicates pregnancy.

When we first devised and published this procedure, pH 4 was chosen for the acidity of hydrolysis because many workers[2-4] using acidities in this range had obtained good yields of hormone, as judged by bio-assay results and isolation of crystalline hormone. Our results with it, as recorded in the original paper,[1] were good. Shortly thereafter, however, papers appeared claiming that much higher degrees of acidity were necessary to obtain good hydrolysis, such as boiling with 15 c.c. of concentrated HCl per 100 c.c. of urine[5] or pH's below 1 and heating under 15 pounds pressure.[6] We then thought that perhaps our low hydrolyzing acidity might have accounted for some of our doubtful results in early pregnancy, and since we could not foresee any harm in using a higher acidity we suggested in a supplement[7] that pH 1 be used. As our own experience with pH 1 increased, we observed that false positives were occa-

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Received for publication, October 8, 1936.
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sionally obtained at this pH, and we, therefore, studied the problem of hydrolyzing acidity in greater detail. The results of these experiments are tabulated in Table I and may be summarized as follows:

TABLE I

URINE	PROCEDURE	RESULTS		
		COLOR TEST	C.C. INJD.	ESTRUS
209* Full-term preg.	pH 4 → 6 c.c. oil		0.1	+
	2nd extn. → 6 c.c. oil		2.0	-
	pH 1 → 6 c.c. oil		1.0	+
			0.5	-
210 3 mo. preg.	pH 4 →	BN† 8.0		
	2nd extn. ½ →	BN 1.0		
	½ → 10 c.c. oil		1.5	-
	pH 1 ½ →	BN 7.75		
	½ → 10 c.c. oil		1.0	+
212 Menopause	pH 4 →	BN 5.9		
	15% → 10 c.c. alc. → 8 c.c. alc.	BN 18.4		
	→ 2 c.c. alc. → 5 c.c. oil		2.0	-
215 1 mo. preg.	pH 4 →	BN 7.0		
	15% ½ →	BN 7.0		
	½ → NaOH sol. fraction	BN 3.3		
	Red residue	BN 3.7		
218 Early preg.	pH 4 → NaOH sol. fr.	FN 21.0		
	→ Residue	FN 9.5		
	15% → 8 c.c. alc.	FN 84.0		
	→ 2 c.c. alc. → 15 c.c. W		5.5	-
219 Full-term preg.	pH 4 → 8 c.c. alc.	FN 60.0		
	→ 2 c.c. alc. → 20 c.c. W		1.0	+
	2nd extn. → 8 c.c. alc.	FN 2.5		
	→ 2 c.c. alc. → 20 c.c. W		3.0	-
	pH 1 → 8 c.c. alc.	FN 63.4		
	→ 2 c.c. alc. → 20 c.c. W		3.0	+
			2.0	-
	15% → 8 c.c. alc.	FN 84.0		
	→ 2 c.c. alc. → 20 c.c. W		3.0	+
			2.0	-

*In Case 209, 1/10 of the 24 hr. specimen was used.

†BN (beta naphthol number) may be converted to FN by multiplying the BN by 6. For a detailed discussion of the use of beta naphthol as a color standard see page 9 of Supplement I.

W, water.

1. As the acidity of hydrolysis is increased, the color, both of the urine itself and of its ether extract, intensifies. For example, at pH 4 the extracts are usually light yellow, at pH 1, orange, and at 15 per cent HCl, often bright red (indirubin?). However, we have convinced ourselves by numerous experiments that the pigment itself does not couple with the diazotized para-nitroaniline and is not responsible for the false positives which may be obtained with strong acid hydrolysis; any color which the pigment may contribute to the final test is its own, that is, it does not increase that color by forming an azo dye as does estrin. We proved this by the following type of experiment. Urines were hydrolyzed by boiling with 15 per cent HCl, and when red ether extracts were obtained (this does not invariably occur) they were washed with 20 per cent Na_2CO_3 as usual and then with 10 per cent NaOH. We found that the NaOH removes the volatile phenols and estrin (as expected) and that the red pigment is alkali insoluble, only a yellow pigment going into the NaOH. When

the ether is now distilled off and the red residue coupled, as in the pregnancy test procedure, negative tests are obtained. Hence, the ether soluble pigments are not responsible for the false positives which are obtained with strong acid hydrolysis.

2. However, as the acidity of hydrolysis increases a compound is produced which is ether soluble, colorless, insoluble in Na_2CO_3 but soluble in NaOH, nonvolatile with steam and capable of coupling with the diazotized para-nitroaniline to give an intensely red azo dye. That is, it has all these properties in common with estrin, but *it is not estrogenic*, and it may be obtained from *any* urine when 15 per cent hydrolysis (less often pH 1) is used. (See Table I, urines 212, 218, 219.) We shall designate this compound as "chromogen" for the present. These positives due to chromogen may be obtained from the light yellow to colorless NaOH washings of the red ether extracts by neutralizing the alkali, distilling off the volatile phenols, re-extracting the chromogen with ether, washing and distilling off the ether and coupling the residue as in the pregnancy test procedure.

We then decided to determine if pH 1 or 15 per cent hydrolysis does actually give any significant further release of hormone subsequent to pH 4 hydrolysis. (It may be noted in passing that all papers advocating a high acidity for estrin ester hydrolysis have reported no detailed studies of the degree of hydrolysis obtained with lower acidities.) This we did by bio-assay and the results can be seen from urines 209, 210, 218, and 219. They indicate that after pH 4 hydrolysis and complete extraction of the deconjugated hormone, subsequent hydrolysis at higher acidities does release still more hormone, but the additional yield then obtained is always much lower than that first obtained at pH 4, usually about one-tenth of that at pH 4.

We therefore conclude that, in the procedure which we have described for the chemical detection of estrin, the most accurate results are to be obtained when the acidity at which the urine is hydrolyzed is not below pH 4. That some further hydrolysis of estrin esters does occur when the acidity of hydrolysis is increased is true, but in our present procedure this further hydrolysis produces, in addition to more free estrin, some chromogen which is confusing in the diagnosis of pregnancy.

REFERENCES

1. Schmulovitz, M. J., and Wylie, H. Boyd: The Chemical Diagnosis of Pregnancy by Detection of Estrin in Urine, J. Lab. & Clin. Med. **21**: 210, 1935.
2. Zondek, B.: Die Hormone des Ovariums und des Hypophysenvorderlappens, Berlin, 1931, Julius Springer.
3. Doisy, E. A., Veler, C. D., and Thayer, S.: The Preparation of the Crystalline Ovarian Hormone From the Urine of Pregnant Women, J. Biol. Chem. **86**: 499, 1930.
4. Marrian, G. F.: The Chemistry of Oestrin—An Improved Method for Preparation and the Isolation of Active Crystalline Material, Biochem. J. **24**: 435, 1930.
5. Smith, G. V. S., and Smith, O. W.: The Quantitative Determination of Urinary Oestrin, Am. J. Physiol. **112**: 340, 1935.
6. Cohen, S. L., and Marrian, G. F.: The Hydrolysis of the Combined Forms of Oestrone and Oestriol Present in Human Pregnancy Urine, Biochem. J. **29**: 1577, 1935.
7. Schmulovitz, M. J., and Wylie, H. Boyd: Mimeographed supplement, Jan. 6, 1936. Note of this supplement and its offer to those interested was appended to the original paper.[1]

RAPID AND ACCURATE METHODS FOR THE DETERMINATION OF UREA NITROGEN IN BLOOD AND URINE BY DIRECT NESSLERIZATION*

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THE constantly increasing demand upon the clinical laboratory for the determination of urea in blood and urine, especially since the introduction of the urea clearance test of Van Slyke as a measure of renal function, makes it paramount that the methods used for these procedures shall be the most simple and accurate, and withal, the most practicable. The inherent possibilities of direct nesslerization methods in meeting these requirements have been generally recognized in the last decade and a score of workers have reported different techniques which are based upon this principle.

Karr,[1] Roe and Irish,[2] Looney,[3] Leiboff and Kahn,[4] and others have described methods of determining urea by direct nesslerization of Folin and Wu tungstic acid filtrates. However, difficulties of one sort or another have been encountered in the application of these procedures which have precluded their general acceptance for routine use.

More recently, Taylor and Blair[5] proposed a method which in principle conforms to one originally suggested by Gruskin.[6] The method in brief is as follows: oxalated blood is incubated with a highly concentrated urease solution prepared in accordance with directions formulated by Sumner.[7, 8] After incubation the proteins are precipitated and the urea is determined colorimetrically by direct nesslerization.

The technique for the determination of urinary urea which is described in this paper has been worked out along the lines followed by Breuer[9] in his modification of Folin and Youngsburg's[10] method.

Since these methods were first introduced into this laboratory more than two years ago, over three thousand urea estimations have been made. Certain modifications have been studied and adopted for the preparation of the urease reagent, and also for the technique of these tests, which have greatly enhanced their value for routine work. The results of these two colorimetric procedures have been carefully checked with the manometric methods of Van Slyke, and, as will be shown, they have proved to be very accurate.

PREPARATION OF UREASE REAGENT

It should be emphasized at the outset that the success of these methods depends primarily upon the exercise of a reasonable amount of care in carrying out the directions for the preparation of the special urease reagent.

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Received for publication, October 16, 1935.
Published with the permission of the Medical Director of the Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the writer.

REAGENTS

1. "Arlco" jack bean meal, a finely powdered fat-free meal obtained from Arlington Chemical Company, Yonkers, New York.

2. 32 Per Cent Acetone Solution: Dilute 158 c.c. of C. P. acetone with distilled water to 500 c.c. in a volumetric flask or graduate. Cool to 22° C. and dilute exactly to the mark.

3. 0.2 Normal Acetic Acid: Approximately 11.3 c.c. of 99 per cent glacial acetic acid is diluted to a liter. Standardize against 0.1 normal NaOH with phenolphthalein as indicator.

4. 32 Per Cent Acetone Phosphate Solution for washing precipitated urease: Dilute C. P. acetone, as for the aqueous solution above, with a mixture containing 143 c.c. of 0.1 M NaOH and 500 c.c. of 0.2 M KH_2PO_4. To prepare the latter solution 13.6 gm. of KH_2PO_4 is diluted to a volume of 500 c.c. In place of KH_2PO_4 one may use 13.8 gm. of $NaH_2PO_4.H_2O$ dissolved in the same volume of water which gives a 0.2 M concentration of this salt. This acetone phosphate solution is chilled so that it will be ice cold when used.

5. C. P. neutral glycerin.

Procedure: Weigh out 100 gm. of jack bean meal and place in a 1 liter beaker. Add 500 c.c. of 32 per cent acetone solution and stir for five minutes to break up the lumps. Filter through a folded filter paper. If the first portion of the filtrate is not clear it should be refiltered. When all or nearly all of the material is on the filter place in the refrigerator. When filtration is complete, 10 c.c. of 0.2 normal acetic acid is added to the acetone filtrate with rapid stirring, and the liquid is allowed to remain overnight on ice. The following morning centrifuge off the precipitate present in the filtrate by using 50 c.c. centrifuge tubes and whirling at moderate speed for eight or ten minutes. When all of the precipitate has been centrifuged off it is washed twice with cold 32 per cent acetone phosphate solution by filling the tubes with this solution, stirring up the precipitate and centrifuging again. The phosphate solution is used to make the urease less soluble in the 32 per cent acetone solution. After the last washing the centrifuge tubes are drained against filter paper until the last trace of acetone is removed, care being taken that the precipitate is not allowed to become dry. Suspend the washed urease precipitate in 5 c.c. of water which has been distilled from a glass condenser. Let stand at room temperature for one or two hours with occasional stirring. In our experience a considerable part of the urease, which is present together with a comparatively large amount of insoluble protein material, dissolves in the water rather slowly. The entire mixture is now transferred to a 15 c.c. centrifuge tube and the bulk of the insoluble matter is centrifuged off. It is neither desirable nor necessary to remove all of this material. The supernatant fluid is decanted into a small graduated cylinder and to it is added an equal volume of C. P. neutral glycerin as a preservative. The resultant solution is slightly turbid in appearance. It should be kept in a small, tightly stoppered bottle in the refrigerator. Under these conditions it will maintain its activity for six months or longer. Tests for ammonia nitrogen with Nessler's solution should give no color. Tests for pentose carbohydrate have always been negative. The directions given above are for 10 c.c. of concentrated urease solution. Larger quantities may be prepared at one time by increasing the amounts of the reagents proportionately.

Determination of Urease Activity: It is always advisable to test the ability of the freshly prepared urease solution to decompose urea into ammonium carbonate. The procedure used is a modification of the methods recommended by Sumner and also by Taylor and Blair. The activity of the concentrated urease preparation is estimated at pH 7.0, which according to Sumner is the optimum pH for the maximum effect of the enzyme, by the use of an urea phosphate solution containing 3 per cent of urea, 6.8 per cent of Na_2HPO_4 and 2.8 per cent of KH_2PO_4. As a substitute for Na_2HPO_4, one may use 17.0 per cent of $Na_2HPO_4.12H_2O$, and for KH_2PO_4, 2.84 per cent of $NaH_2PO_4.H_2O$. The urea phosphate solution is preserved with toluene or chloroform and renewed every few weeks.

Method: 25 c.c. of urea phosphate solution is placed in a small Erlenmeyer flask. One drop of urease reagent is added from a 1 c.c. graduated pipette which delivers 22 drops per c.c. The flask is stoppered and placed in a water-bath at 45 or 50° C. for fifteen minutes. At the end of this interval transfer exactly 1 c.c. of the mixture to a 200 c.c. volumetric flask

containing about 150 c.c. of distilled water. A standard is prepared at the same time containing 5 mg. of ammonia nitrogen and 1 c.c. of urea phosphate to balance that in the unknown. To each flask add 20 c.c. of Nessler's solution. Dilute to the marks, mix and compare in the colorimeter, setting the standard at 10 mm.

Calculation:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 125 = \text{mg. urea N formed from 25 c.c. of 3 per cent urea phosphate per drop of urease.}$$

Although this method is by no means exact, it is easily performed and will indicate the relative efficiency of the urease preparation. Under these conditions the urea hydrolyzing power of a large number of extracts prepared in this laboratory has ranged from 50 to 110 mg. of urea nitrogen per drop. These differences in urease activity have been attributed largely to variations in the enzyme content of the jack bean meal which has been adequately demonstrated by Sumner. However, variations within this range are perfectly satisfactory as far as the use of the urease solutions for clinical work is concerned.

METHOD OF PROCEDURE FOR BLOOD UREA

Reagents—

Concentrated urease solution prepared as described.

10 per cent sodium tungstate (Folin and Wu[11]).

⅔ normal sulphuric acid.

Nessler's solution (Koch and McMeekin[12]).

Standard ammonium sulphate solution containing 0.1 mg. of ammonia nitrogen per cubic centimeter.

Procedure.—Pipette 5 c.c. of oxalated blood into a 20 volume Erlenmeyer flask. Dilute the blood with 7 volumes of water and mix. With a 1 c.c. pipette add 1 drop of the special urease reagent. Mix by agitating slightly, close the mouth of the flask with a stopper and let stand at room temperature for fifteen minutes. Add slowly with stirring 1 volume of 10 per cent solution of sodium tungstate and mix. Then add slowly with stirring 1 volume of ⅔ normal sulphuric acid. Stopper the flask, mix the contents well and allow to stand for a few minutes. The protein precipitate is then removed by filtration.

The filtrate obtained as described can be applied to the determination of other blood constituents in accordance with the Folin-Wu procedures. Small amounts of blood, 2 or 3 c.c., can, of course, be used with the same degree of accuracy when the urea nitrogen result only is desired. When more than 5 c.c. of blood is to be analyzed more urease reagent is added at the rate of 1 drop for each 5 c.c. or less of additional blood used. By carefully controlled tests, it has been proved that the addition of seven volumes of water to the blood before treatment with urease, instead of two volumes as recommended by Taylor and Blair, has no demonstrable effect on the natural buffering properties of the blood for the urease, and the accuracy of the results is not affected even with specimens of high urea content. On the other hand, this further dilution is a definite advantage in preventing glycolysis in specimens to be examined for sugar content also, and, by eliminating the unnecessary step of adding the five volumes of water after the period of enzyme action, the time-saving element is considerable when a large number of specimens are to be analyzed. It is

important also to note here that incubation at 45° C. for the action of urease, as advised by the writers mentioned above, is not essential to attain the full effect of the urease under the conditions of the test, but may result in volatilization of ammonia, especially when the concentration of blood urea is high.

For the urea nitrogen determination, 5 c.c. of the filtrate obtained as described is pipetted into a 25 c.c. graduated tube or volumetric flask, 15 c.c. of water is added followed by 2.5 c.c. of Nessler's solution and dilution to the mark. A standard is prepared at the same time as follows: pipette 4 c.c. of the ammonium sulphate standard (1 c.c. = 0.1 mg. N) into a 100 c.c. volumetric flask, add about 50 c.c. of water, 10 c.c. of Nessler's solution, and dilute to volume. If a specimen of high urea content is anticipated a more accurate comparison can be made by diluting with water to a volume in which the color approaches that of the standard, using a proportional amount of Nessler's solution.

No trouble is experienced in this method with the formation of turbid solutions following nesslerization, the unknown solutions remaining perfectly clear for thirty minutes or longer. In this connection it should be stated that better results will be obtained if the modified Nessler's reagent of Koch and McMeekin is used. This reagent is essential for the procedure with urinary urea. In the blood determination, the color match with the ammonium sulphate standard is excellent, a good comparison in practically all cases being possible with the standard set at 20 mm. Occasionally a blood specimen of low urea content is encountered which yields a color with a slightly greenish tinge. In such instances a better match can be obtained by setting at 15 mm.

Calculation of Results—

$$\frac{\text{Reading of Standard (usually 20)} \times 20}{\text{Reading of Unknown}} = \text{mg. urea N per 100 c.c. of blood.}$$

NOTE: The equivalent of 0.5 c.c. of blood is used; the standard contains 0.4 mg. nitrogen; the volume of the standard is four times the volume of the unknown. The factor 20 is obtained as follows:

$$\frac{0.4}{4} = 0.1 \text{ mg. urea N in 0.5 c.c. of blood, or}$$

20 mg. urea N in 100 c.c. of blood.

PROCEDURE FOR URINARY UREA NITROGEN

Reagents—

Concentrated urease solution prepared as described.

Permutit powder (Folin).

Phosphate buffer solution: 5 gm. of KH_2PO_4, or, 5.07 gm. of NaH_2PO_4. H_2O and either 5 gm. of Na_2HPO_4. $12H_2O$ or 2 gm. of anhydrous Na_2HPO_4 per 100 c.c.

Nessler's reagent (Koch and McMeekin).

Standard ammonium sulphate solution containing 0.1 mg. of ammonia nitrogen per c.c.

Procedure.—Transfer accurately 1 c.c. of urine to a 100 c.c. Erlenmeyer flask and add enough water from a buret to dilute the urine exactly to 40 c.c.

If the specific gravity of the specimen is very low make this dilution 1-20. Two grams of washed permutit powder measured approximately by volume are placed in a 15 c.c. graduated centrifuge tube and the tube is filled with the diluted urine. The mixture is shaken gently for five minutes to remove preformed ammonia. Centrifuge until clear. Transfer 2 c.c. of the clear supernatant liquid to another 15 c.c. graduated centrifuge tube, add exactly 2 c.c. of the phosphate buffer and 1 drop of the urease reagent. Stopper the tube and

TABLE I

COMPARISON OF BLOOD UREA NITROGEN DETERMINATIONS

SPECIMEN NO.	VAN SLYKE METHOD	PROPOSED COLORIMETRIC METHOD	DIFFERENCE MG.	DEVIATION
	MG. UREA N PER 100 C.C.	MG. UREA N PER 100 C.C.		PER CENT
1	7.7	8.3	+0.6	7.8
2	9.4	9.9	-0.5	5.3
3	12.5	11.6	-0.9	7.7
4	13.9	15.4	+1.5	10.6
5	15.5	16.1	+0.6	3.8
6	23.8	24.0	+0.2	0.8
7	38.5	39.0	+0.5	1.3
8	50.7	49.1	-1.6	3.2
9	98.7	97.5	-1.2	1.4
10	130.3	133.0	+2.7	2.0
11	178.0	181.0	+3.0	1.6
12	260.0	258.0	-2.0	0.7

TABLE II

COMPARISON OF URINE UREA NITROGEN DETERMINATIONS

SPECIMEN NO.	VAN SLYKE METHOD	PROPOSED COLORIMETRIC METHOD	DIFFERENCE MG.	DEVIATION
	MG. UREA N PER 100 C.C.	MG. UREA N PER 100 C.C.		PER CENT
1	264	265.9	+1.9	0.7
2	358	355.0	-3.0	0.8
3	519	526.0	+7.0	1.3
4	636	634.0	-2.0	0.3
5	678	681.0	+3.0	0.4
6	733	727.0	-6.0	0.8
7	928	932.0	+4.0	0.4
8	1134	1141.0	+7.0	0.6
9	1217	1219.0	+2.0	0.1
10	1381	1377.0	-4.0	0.2

mix the contents by holding the tube at the upper end and tapping the lower end with the finger. Allow to stand at room temperature for fifteen minutes. Dilute to 10 c.c. and mix. Transfer 5 c.c. of the clear fluid to a 25 c.c. graduated tube or volumetric flask, add 15 c.c. of water and 2.5 c.c. of Nessler's solution. Dilute to 25 c.c. ,or, if necessary, this dilution may be carried further to a volume comparable with the standard. The standard used is the same as that prepared for blood, that is, 4 c.c. of standard ammonium sulphate (1 c.c. = 0.1 mg. N) is pipetted into a 100 c.c. volumetric flask, 50 c.c. of water is added followed by 10 c.c. of Nessler's solution and dilution to volume.

Calculation—

$$\frac{\text{Reading of Standard (usually 20)} \times 400}{\text{Reading of Unknown}} = \text{mg. urea N per 100 c.c. of urine.}$$

NOTE: The equivalent of 0.025 c.c. of urine is used; the standard contains 0.4 mg. nitrogen; the volume of the standard is four times the volume of the unknown. The factor 400 is obtained as follows:

$$\frac{0.4}{1} = \begin{array}{l}\text{0.1 mg. urea N in 0.025 c.c. of urine, or} \\ \text{400 mg. urea N in 100 c.c. of urine.}\end{array}$$

EXPERIMENTAL DATA

The colorimetric methods herein described were compared in a series of determinations performed on blood and urine specimens with the gasometric urease procedures of Van Slyke.[13] The data for these results are presented in Tables I and II. The figures shown for both colorimetric and gasometric methods represent the average of duplicate analyses in all cases.

SUMMARY

Methods are described for the determination of urea in blood and urine which are rapid and accurate. Both of these methods depend on the use of a concentrated urease reagent the preparation of which is also given in detail.

REFERENCES

1. Karr, W. G.: A Method for the Determination of Blood Urea Nitrogen, J. LAB. & CLIN. MED. **9**: 3, 1924.
2. Roe, J. H., and Irish, O. J.: An Accurate Method for the Estimation of Urea in Blood and Urine by Direct Nesslerizaton, J. LAB. & CLIN. MED. **11**: 1087, 1926.
3. Looney, J. M.: The Determination of Blood Urea Nitrogen by Direct Nesslerization, J. Biol. Chem. **88**: 189, 1930.
4. Leiboff, S. L., and Kahn, B. S.: A Rapid and Accurate Method for the Determination of Urea in Blood, J. Biol. Chem. **83**: 347, 1929.
5. Taylor, W. F., and Blair, W. M.: An Improved Method for the Determination of Blood Urea Nitrogen by Direct Nesslerization, J. LAB. & CLIN. MED. **17**: 1256, 1932.
6. Gruskin, B.: A New and Shorter Method for the Determination of Urea and Sugar in the Blood, J. LAB. & CLIN. MED. **10**: 233, 1924.
7. Sumner, J. B.: Note. The Recrystallization of Urease, J. Biol. Chem. **70**: 97, 1926.
8. Sumner, J. B., and Hand, D. B.: Crystalline Urease. II, J. Biol. Chem. **76**: 149, 1928.
9. Breuer, M. J.: A Method for Urinary Urea, J. LAB. & CLIN. MED. **16**: 1221, 1931.
10. Folin, O., and Youngsburg, G. E.: Note on the Determination of Urea in Urine by Direct Nesslerization, J. Biol. Chem. **38**: 111, 1919.
11. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**: 81, 1919.
12. Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. **46**: 2066, 1924.
13. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. II, Baltimore, 1932, Williams & Wilkins.

HIPPURIC ACID ELIMINATION AS A TEST FOR LIVER FUNCTION*

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THE functions of the liver are many and varied, which makes the determination of the functional state of this organ a most difficult task. It is impossible for any one test to make such a determination and hence several available tests have to be used. We wish to present our observation of a test that can be easily performed, and which we feel offers a great deal of information when used in conjunction with other tests. This test is that of hippuric acid synthesis as described by Quick.[1]

Studies on the synthesis of hippuric acid have been carried on for a long time. This synthesis was first discovered by Wöhler in 1824, yet it remained for Bunge and Schmeidenberg fifty-three years later to show that if the kidneys of dogs were perfused with benzoic acid and glycine, hippuric acid was formed. From this work these men concluded that the kidney was the site of this synthesis and hence attempted to establish the test as one for kidney function study. This work was further carried on by Janevald and Stokvis who found that this synthesis was diminished or absent in diseases of the kidney especially in parenchymatous nephritis.

From this time on, there has been considerable controversy as to the site of the formation of hippuric acid. Weyl and Anrep[2] were opposed to the kidney theory. In 1857, Kühne and Hallwachs concluded that the synthesis occurred in the hepatic vessels in the presence of glycocolic acid. In 1911, Friedman and Tachau found that the liver took part in this synthesis. They isolated hippuric acid after perfusing the liver of a rabbit with blood containing benzoic acid, but no glycine, and concluded that the glycine was present in the liver in response to the benzoic acid.

Kingsbury and Bell found hippuric acid in the tissues of nephrectomized dogs after sodium benzoate and glycine were injected. In 1918, Lachner, Levinson, and Morse[3] showed that in dogs the excretion of hippuric acid varied with the condition of the liver, the kidney remaining constant, and they concluded that pathologic involvement of the liver should be recognized by this means. They poisoned dogs with hydrazine sulphate and found the synthesis greatly diminished and yet there was no damage to the kidney. Delprat and Whipple in their experiments on liver necrosis due to chloroform found that the synthesis of hippuric acid is much delayed. Morgulis and others,[4] in 1923, showed that extensive injury to the liver is responsible for the failure of the formation of glycine.

More recently (1925), Bryan,[5] in attempting to use this test as one of kidney function, showed that hepatic damage in dogs produced a decrease in

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Received for publication, October 19, 1936.

the amount of available glycine in the liver, and that the synthesis in other organs as the kidney was reduced or absent. The excretion of the free benzoic acid indicated the presence of liver damage, and he accounted for it as being due to the inability of the liver to mobilize glycine. Several years later, the experiments of Bunge and Schmeidenberg[6] were repeated by Snapper, Grünbaum, and Neuberg.[7] Kingsbury and Bell then perfused two human kidneys soon after their surgical removal and demonstrated definite amounts of hippuric acid in the perfused blood to which sodium benzoate and glycine were added.

In 1933, A. J. Quick[8] revealed that Bunge and Schmeidenberg[6] failed to find hippuric acid after injecting sodium benzoate and glycine into dogs in which the renal blood vessels were tied, but they were able to find it when the ureters were ligated and the vascular supply of the kidney was left intact. He also showed that no hippuric acid was detected in the blood or liver of nephrectomized dogs that were given sodium benzoate and glycine, and was never found when the ureter was ligated. On feeding sodium benzoate to nephrectomized rabbits, hippuric acid was found in the blood, liver, and muscles. From the above work, Quick[9] concluded that the synthesis of hippuric acid is effected by enzyme, hippuricese, which in a dog is apparently present in the kidney only.

To confirm the liver site, Lewis showed that if a cannula was inserted in the common bile duct of a rabbit the synthesis of hippuric acid occurred after the exclusion of bile from the intestines. Clinically, Kingsbury and Swanson[10] showed that this test was useless as one of the kidney function for in known cases of kidney damage, normal amounts of hippuric acid are excreted.

In regard to the mechanism of this synthesis, it is known that hippuric acid is one of the end-products of protein metabolism and is a combination product of glycine and benzoic acid. The benzoic acid arises from the aromatic products of vegetable foods or from phenylalanin. The origin of glycine is much more complicated. The available sources are: (1) synthesis; (2) preformed glycine which includes the glycine liberated by the catabolism of body protein and present in the food. Other substances, besides benzoic acid, which may give rise to an increased output of hippuric acid are: cinnamic acid, toluene, quinic acid, and fruits and vegetables, especially cranberries and prunes. However, Quick[11] proved that 6 gm. of either quinic acid or prunes will yield only 0.15 gm. of hippuric acid, hence these are not factors. In carnivorous animals, it was found that the degree of hippuric acid formation after the introduction of benzoic acid is much lower than in herbivorous animals and the free benzoic acid output was much greater than that combined with glycine. In dogs, the detoxication of benzoic acid occurs only in a small part by the formation of hippuric acid and to a considerable extent by the formation of other reducing substances as glycuronic acid, monobenzoate. In man, benzoic acid is almost completely excreted as hippuric acid.

A diet rich in proteins enables human beings to transform more benzoic acid to hippuric acid according to Lewinski.[12] Cohen,[13] working with rabbits, noted that feeding proteins and their glycine-yielding decomposition products counteracted the toxic effect of benzoic acid. Griffith and Lewis,[14] also working with rabbits, noted that the excretion of hippuric acid is increased when glycine

is fed with the sodium benzoate. They showed that other proteins besides glycine do not produce this increased output. Thus they concluded that the ordinary reactions of protein metabolism do not form the precursors of glycine.

Lewinski[12] found that a man could completely convert 20 gm. of benzoic acid in twelve hours without any ill-effects. Lewis gave six 10 gm. of which 85 to 90 per cent were excreted as hippuric acid in five or six hours. From these experimental data, Bryan[15] concluded that normal people can convert 25 gm. of sodium benzoate to hippuric acid without any toxic symptoms. If larger amounts of benzoic acid are given this can also be converted by feeding additional glycine.

The fundamental factor governing and limiting formation of hippuric acid is the rate of synthesis of glycine by the body. The body does not have a store of preformed glycine, but produces it in response to stimulation by benzoic acid. Despite the amount of benzoic acid administered, the hourly excretion of hippuric acid rises to a maximum and remains constant until all but a small portion is eliminated. If additional glycine is given, then the hourly excretion rises. The body is able to produce between 0.55 and 9.70 gm. of glycine per hour which was computed by Quick. These figures well agree with those of Bignami who found that the available glycine was 0.009 gm. per kilo. Glycine may also be synthesized from carbohydrate derivatives, and when the deficiency in available carbohydrates occurs as indicated by the presence of a ketosis, then the synthesis of glycine is diminished or absent.

TABLE I

HIPPURIC ACID ELIMINATION AS A TEST FOR LIVER FUNCTION

DIAGNOSIS	CASES STUDIED
Cases with definite liver pathology	18
Cases with gallbladder disease	7
Cases with cardiovascular renal disease	25
Cases with miscellaneous diseases as controls	50
Total cases studied	100

TABLE II

HIPPURIC ACID EXCRETION TEST IN MISCELLANEOUS CASES AS CONTROLS

	SUBJECT	AGE	DIAGNOSIS	1ST HR.	2ND HR.	3RD HR.	4TH HR.	TOTAL
1.	M. C.	38	Syphilitic osteo. spine	1.05	0.84	0.83	1.35	4.07
2.	P. E.	16	Herniotomy	0.63	1.66	1.11	0.90	4.30
3.	J. J.	24	Bismuth poisoning	0.67	1.31	1.35	0.42	3.75
4.	M. W.	35	Hydatidiform mole	0.29	0.60	0.82	1.56	3.27
5.	D. B.	25	Pneumonia	1.57	0.97	0.43	0.46	3.43
6.	L. P.	54	Hodgkin's	0.58	1.11	1.11	1.59	4.39
7.	I. C.	56	Carcinoma of tail pancreas	0.60	0.97	1.17	1.33	4.07
8.	M. C.	43	Appendiceal abscess	1.02	1.17	0.56	1.19	3.94
9.	M. G.	39	Renal calculi	0.29	0.88	0.97	0.92	3.06
10.	H. W.	42	Chronic osteo.	0.48	1.06	1.36	0.92	3.82

The technique of the test as simplified by Quick is as follows: Five and nine-tenths grams of sodium benzoate (equivalent to 5 gm. of benzoic acid) dissolved in 30 c.c. of water are administered one hour after breakfast consisting of coffee and toast. The patient is then given one-half glass of water. Immediately

after taking the drug the patient voids, and then collects complete hourly specimens for four hours. These are preserved with toluene, and hippuric acid determined in each specimen. Each hour specimen is measured, transferred to a small beaker and acidified with concentrated hydrochloric acid until acid to Congo red; 1 c.c. of the acid is usually sufficient. The solution is vigorously stirred until the precipitation of hippuric acid is complete, and then is allowed to stand for one hour at room temperature. The precipitate is filtered off on a small Büchner filter plate, washed with a small quantity of cold water, and allowed to air dry. The hippuric acid thus obtained is either weighed (to the second decimal place, which is usually sufficient) or titrated with 0.2 normal sodium hydroxide, phenolphthalein being used as indicator. To obtain the total hippuric acid, one must add to the amount thus obtained the calculated quantity

TABLE III

HIPPURIC ACID EXCRETION TEST IN LIVER DAMAGE

	SUBJECT	AGE	DIAGNOSIS	1ST HR.	2ND HR.	3RD HR.	4TH HR.	TOTAL
1.	I. W.	67	Met. ca. of liver	0.58	0.58	0.12	0.12	1.40
2.	M. A.	27	Catarrhal jaundice	0.41	0.33	0.39	0.29	1.42
3.	C. J.	64	Cirrhosis of liver	0.57	0.32	0.59	1.17	2.65
4.	H. A.	25	Arsphen. hepatitis	0.61	0.61	0.69	0.43	2.34
5.	P. M.	52	Met. ca. of liver	0	0.68	0.66	1.52	2.86
6.	A. F.	35	Biliary cirrhosis	0.61	0.27	0.66	0.28	1.82
7.	L. S.	50	Met. ca. pancreas	0.41	0.30	0.30	0.37	1.38
8.	H. M.	45	Met. ca. of liver	0.37	0	0.84	0.83	2.04
9.	R. N.	23	Arsphen. hepatitis	0.09	0.15	0.16	0.11	0.51
10.	G.	27	Generalized T.B. liver, etc.	0	0.31	0.47	0	0.78

TABLE IV

HIPPURIC ACID EXCRETION TEST IN GALLBLADDER DISEASE

	SUBJECT	AGE	DIAGNOSIS	1ST HR.	2ND HR.	3RD HR.	4TH HR.	TOTAL
1.	R. P.	60	Cholecystitis	0.68	0.80	1.22	0.83	3.73
2.	S. S.	48	Common duct obstruction	0.75	0.74	1.16	1.24	3.89
3.	E. L.	19	Postcholecystectomy syndrome	0.82	0.80	1.09	0.90	3.71
4.	F. L.	37	Cholecystitis	0	1.82	0	1.63	3.45
5.	M. W.	30	Cholecystitis	0.53	0.79	0.81	1.41	3.54
6.	T. B.	41	Cholelithiasis	0.53	0.83	0.80	1.00	3.16

TABLE V

HIPPURIC ACID EXCRETION TEST IN CARDIOVASCULAR RENAL DISEASE

	SUBJECT	AGE	DIAGNOSIS	1ST HR.	2ND HR.	3RD HR.	4TH HR.	TOTAL
1.	B. F.	58	Hypertension	0.31	0.46	0.62	1.34	2.73
2.	R. F.	58	Cardiac	0.62	0.97	2.18	0.75	4.42
3.	B. S.	64	Cardiac decomp.	0.07	0.58	0.25	0.34	1.24
4.	T. McN.	65	Cardiac decomp.	0.31	0.41	0.12	0.0	0.84
5.	E. S.	59	Cardiac decomp.	0.63	1.44	0.70	0.0	2.77
6.	J. M.	54	Cardiac decomp.	0.56	0.59	1.24	0.0	2.39
7.	J. S.	62	Cardiac	0.82	1.12	1.03	1.69	4.66
8.	F. A.	44	Cardiac	0.79	0.62	1.30	1.44	4.15
9.	G. P.	39	Cardiac decomp.	0.15	0.28	0.39	0.90	1.72
10.	S. G.	60	Cardiac decomp.	0.46	0.34	0.50	0.23	1.53

TABLE VI

COMPARATIVE CHART WITH SEVERAL OTHER LIVER FUNCTION TESTS

NAME	AGE	DIAGNOSIS	ICTERUS INDEX	BROMSULPH.	GALACTOSE	VAN DEN BERGH	HIPPURIC ACID	UROBILIN IN URINE
McG.	55	Portal cirrhosis	plus 18	II or 40% ret.		I—delayed ft. pos. D—imm. st. pos.	0.88	
F.	66	Hodgkin's sarcoma	plus 4	0 or no ret.	0.5 gm.	D—imm. ft. pos. I—neg.	2.15	++---- +----- -----
M.	43	C. D. Obst.	plus 80 plus 18	II or 30% ret.		D—imm. st. pos. I—st. delayed mod. pos.	3.52	----
G.	59	Ch. cholecystitis	plus 6.4		1.44 gm.	D—delayed ft. pos. I—imm. ft. pos.	2.46	--+++-- +-+--- ---+
G.	27	Generalized T.B. liver, etc.	plus 90	III or 60% ret.	0	D—imm. st. pos. I—imm. st. pos.	0.78	
W. I.	67	Ca. of liver	plus 100			D—imm. st. pos. I—imm. st. pos.	1.4	
	2 wk.		plus 27	II or 40% ret.		D—imm. st. pos. I—del. ft. pos.	2.64	---+
A.	30	Syphilis of liver	plus 2	0 or 100% ret.	1.09 gm.	D—0 I—0	2.77	
S.		Portal cirrhosis	plus 13	I or 20% ret.	0.1 gm.	D—del. mod. pos. I—imm. mod. pos.	0.96	
C.	52	Portal cirrhosis (varices)	plus 6	0 or no ret.	0.1 gm.	D—neg. I—neg.	3.11	

TABLE VI—CONT'D

NAME	AGE	DIAGNOSIS	ICTERUS INDEX	BROMSULPH.	GALACTOSE	VAN DEN BERGH	HIPPURIC ACID	UROBILIN IN URINE
B.	21	Catarrhal jaundice	50			D—imm. st. pos. I—imm. st. pos.	0.65	
S.	70	Portal cirrhosis	8			D—del. mod. pos. I—del. ft. pos.	1.27	
S.	70	Ca. of G.B. with C. duct obstruction	176	IV or 100% ret.	1.6 gm.	D—imm. st. pos. I—imm. st. pos.	0.00	
A.M.	27	Catarrhal jaundice	plus 100 plus 40 plus 30				1.57	
J.C.	64	Cirrhosis of liver	plus 15 plus 2			D—imm. st. pos. del. mod. pos. I—imm. st. pos. del. ft. pos.	2.67	
A.	25	Arsph. hepatitis	plus 44	III or 80% ret.			2.24	
F.	35	Biliary cirrhosis	plus 40 plus 30 plus 20 plus 15			D—imm. st. pos. I—imm. st. pos.	1.82	
M.H.	45	Ca. of liver (secondary)			1 gm.		2.03	+ in 1:20
R.N.	23	Arsph. hepatitis	plus 64	III or 80% ret.	1.1 gm.	D—imm. st. pos. I—imm. ft. pos.	0.51	++++ ---- ----

remaining in solution; 100 c.c. of urine will dissolve 0.33 gm. of hippuric acid. In case a specimen exceeds 125 c.c. it should be slightly acidified with acetic acid and concentrated on the water-bath to about 50 c.c. before precipitating the hippuric acid. The results are best expressed in terms of benzoic acid. To convert hippuric acid to benzoic, one multiplies by 0.68.

Toxic symptoms of benzoic acid are of two kinds: (1) Those due to local irritation of the gastrointestinal mucosa, with nausea, vomiting, diarrhea, anorexia; and (2) central nervous system symptoms with headache, tinnitus, vertigo, giddiness, and reflex vomiting.

Quick was the first to use this test as one of liver function tests. Since his work, there have only been two other articles dealing with the use of hippuric acid in liver function test: one by Vaccaro, and the other by Hirsheimer.

We have used this test in 50 control patients, those who came into the hospital for conditions other than those referable to the liver and biliary systems, and found that the normal excretion at the end of four hours was three or more grams. In a group of 24 cases involving the cardiovascular system, there was a normal excretion in the absence of heart failure, whereas in that group of patients where there were signs of congestive failure, there was a diminished output of hippuric acid. In 18 patients with disease of the liver or biliary system, we used this test in conjunction with the icterus index, bromsulphthalein, galactose tolerance, van den Bergh, and daily urobilinogen in the urine. We found in cases in which there was damage to the liver there was a decided lower output. The test was very valuable in differentiating jaundice due to hepatitis or obstruction, for those cases of obstruction gave a normal output whereas in those due to hepatitis the excretion was lowered. The test also served as a guide to therapy and prognosis; for as a functional state of the liver improved, the excretion of hippuric acid increased. Portal cirrhosis, biliary cirrhosis, and malignancy of the liver or biliary system all gave lowered outputs. Seven cases of cholecystitis without damage to the liver gave normal results, hence the test is of value to the surgeon to determine the functional state of the liver prior to operation.

Although our series of cases is small, we, nevertheless, feel that this test is a valuable adjunct and recommend that it be used more extensively.

SUMMARY AND CONCLUSIONS

Hippuric acid elimination test was carried in 100 clinical cases including positive damaged liver, cholecystitis and cardiorenal diseases. From this limited number of cases we came to the conclusion that:

1. This test is very valuable to differentiate intrahepatic and extrahepatic jaundice. In liver damage the elimination is very low, while in acute obstruction, the elimination is within normal limits. However, in chronic obstruction where there is liver damage, the differential diagnosis will be impossible with this test.

2. The test has no value in patients with advanced cardiac decompensation with natural result of passive congestion of the liver the elimination if very low.

3. In advanced kidney diseases, the test has no practical value, since there is hardly any elimination of the hippuric acid. Furthermore, it is counter-indicated in such conditions.

4. Repeated estimations of the elimination of hippuric acid are of value in prognosis of liver damage. When the elimination comparatively increased, the prognosis is generally good.

REFERENCES

1. Quick, A. J.: Site of Synthesis of Hippuric Acid and Phenyl Aceturic Acid in the Diagnosis, J. Biol. Chem. **96**: 73, 1932.
2. Weyl and Anrep: Ueber des Flusscheidung der Hippunseus Benzoe Saure Wahrend des Fiebers, Ztschr. f. Physiol. Chem. **4**: 169, 1880.
3. Lachner, Levinson, and Morse: Rôle of Liver in Hippuric Acid Synthesis, Biochem. J. **12**: 184, 1918.
4. Morgulis, Pratt, and Jahr: Hippuric Acid Synthesis as a Test of Renal Function, Arch. Int. Med. **31**: 116, 1923.
5. Bryan, A. W.: Clinical and Experimental Studies on Sodium Benzoate, J. Clin. Investigation **2**: 1, 1925.
6. Bunge and Schmeidenberg: Ueber die Bildung der Hippursaüre, Arch. f. Exper. Path. u. Pharmakol. **6**: 233, 1877.
7. Snapper, Grunbaum, and Neuberg: Ueber die Hippursaüre Synthese in der Überlebenden Niere von Verscheidenen Tiergattunger, Auch von Menschen, Biochem. Ztschr. **145**: 40, 1927.
8. Quick, A. J.: Synthesis of Hippuric Acid, Am. J. M. Sc. **185**: 630, 1933.
9. Quick, A. J.: Conjugation of Benzoic Acid in Man, J. Biol. Chem. **92**: 60, 1931.
10. Kingsbury and Swanson: Synthesis and Elimination of Hippuric Acid in Nephritis. A New Renal Function Test, Arch. Int. Med. **28**: 220, 1921.
11. Quick, A. J.: Factors Influencing Excretions of Uric Acid, J. Biol. Chem. **98**: 157, 1932.
12. Lewinski: Ueber die Grenzen der Hippursaüre und Hung Beim Menschen, Arch. f. Exper. Path. u. Pharmakol. **58**: 397, 1908.
13. Cohen, R.: Ueber den Glykokollvorrat jes Thierischen Organismus, Braunshweig, p. 397, 1901.
14. Griffith and Lewis: Synthesis and Rate of Elimination of Hippuric Acid After Administration of Benzoates, J. Biol. Chem. **57**: 697, 1923.
15. Bryan, A. W.: Clinical and Experimental Studies in Sodium Benzoate, J. Clin. Investigation **2**: 1, 1925.

A TIGHT GAS MASK*

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IN MANY experiments we wish to use a respiratory mask which allows no leakage, instead of resorting to a tracheotomy, which necessitates sacrificing the animal, or an intratracheal catheter, which entails the use of an anesthetic.

The following device is simple and may be used with great success. It consists of a Shaw rubber collar, such as is used in the Drinker artificial respiration machine, attached, as shown in the figure, to any type of chamber large

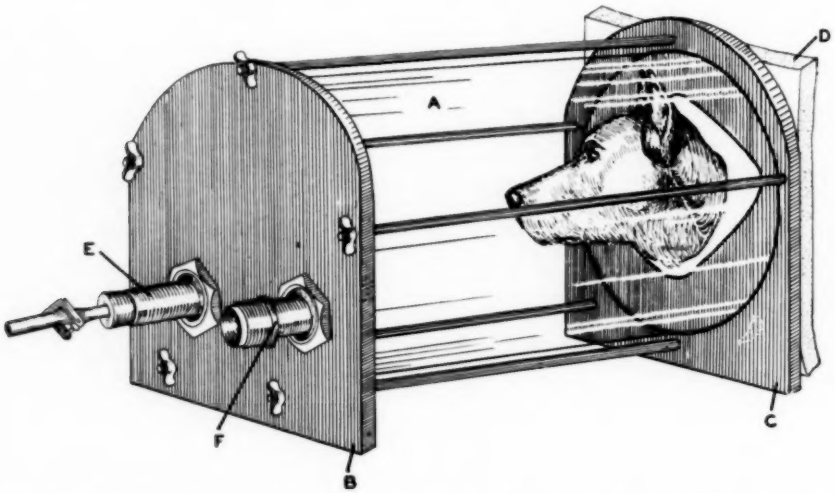

Fig. 1.—The apparatus consists of a glass cylinder (*A*) clamped by means of rods against two metal plates (*B* and *C*) after a rubber gasket has been inserted at each end. The Shaw collar (*D*) is clamped to one of these plates. *E* and *F* are tubes for circulating gas, taking samples, etc. The dead space is unfortunately greatly exaggerated in this drawing.

enough to contain the animal's head. If a dog's neck is shaved and the head forced through the collar into the chamber, an air-tight compartment can be obtained into which or from which gases may be added or subtracted at will. The animal will lie perfectly quiet in such a mask, and, if this is made of glass, the onset of effects produced by breathing any gas can be observed without any disturbance having been caused by the mask.

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Received for publication, October 1, 1936.

SOME SIMPLE APPARATUS FOR THE CULTURE OF SURGICAL MAGGOTS USED IN THE TREATMENT OF CHRONIC OSTEOMYELITIS AND OTHER SUPPURATIVE INFECTIONS*

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THE apparatus described below was devised through a study of an economical method of culturing the maggot of the blowfly for surgical use. It is simple and inexpensive, and has proved very satisfactory. It is hoped that others may find this apparatus useful not only in the culture of surgical maggots but also in general studies of the blowfly or other flies.

1. PUPAE COLLECTOR

By means of the pupae collector (Fig. 1) pupae can be separated rapidly from the sand in which they form (Fig. 2). The collector consists of wire and

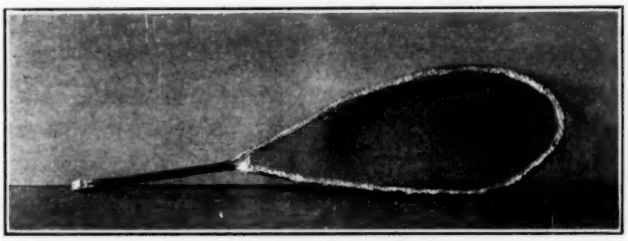

Fig. 1.—Pupae collector.

door screen and is made as follows: First, the wire is shaped to simulate a spoon, the two ends being fastened together with a small piece of wire and covered with liquid solder to make the joint firm. Next, the screen is cut so that a half inch border extends beyond the periphery of the spoon. Then, the half inch border of the screen is folded over the periphery of the spoon, trimmed down, and coated with liquid solder, the solder serving not only to fasten the screen to the wire frame but also to prevent cutting by the jagged edges of the screen.

2. BROOD LARVAL RECEPTACLE

The brood larval receptacle (Fig. 3) consists of a one-pound coffee can, a glass crystallizing dish (or similar container) for a feeding vessel, and some sand for the pupation of larvae. This device requires no renewal of parts and has proved very satisfactory as an all-round brood receptacle. To facilitate a free circulation of air through the receptacle, which is necessary in brood production, the greater portion of the cover of the can is cut out and replaced with

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Received for publication, November 1, 1936.

a 60-mesh brass wire cloth* which is fastened down with liquid solder. The close fit of the cover over the top of the can prevents larvae from making their way between the can and the cover where they would be crushed when the can is opened, as is the case when jars with grooved metal covers are used.

In preparing the receptacle for use, the meat with the eggs is placed into the feeding dish, and the feeding dish is placed into the can which is then covered. As soon as the larvae appear full grown (approximately 15 mm. in

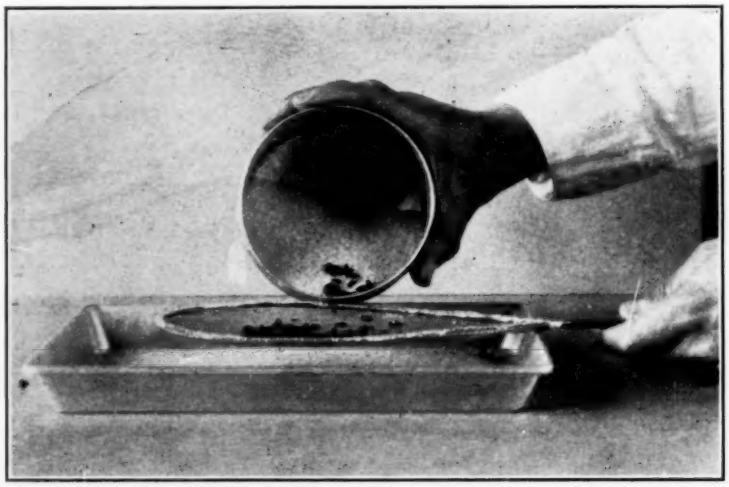

Fig. 2.—Manner in which pupae are collected. The sand containing the pupae is sifted through the collector.

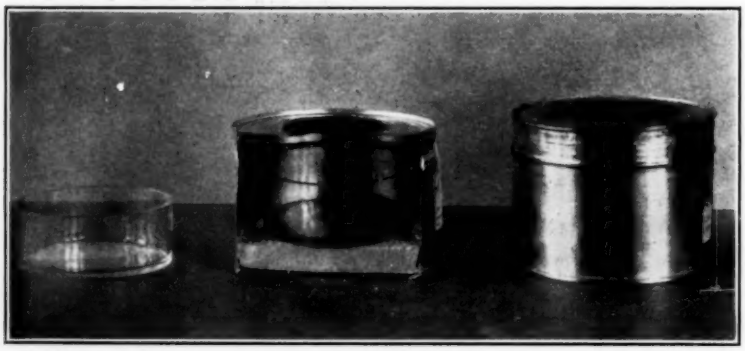

Fig. 3.—Brood larval receptacle. The sectioned receptacle shows the arrangement.

length) about an inch of sand is added to the can for pupation. When nearly all the larvae have migrated from the feeding dish, the dish and the remaining food are removed, and the larvae are left to pupate. Sand is frequently added to the receptacles when they are being prepared for use, but this practice was not found desirable in this laboratory because larvae often leave their food before they are mature; and if sand is present they will enter it, pupate prematurely, and develop as a result into small flies with low egg-laying capacity. When

*A finely perforated section of isinglass or bleached x-ray film may also be used.

sand was not added to the receptacles until the larvae appeared full grown, it was observed that many of the larvae that left their food prematurely returned to it, resumed feeding, and reached maturity normally.

3. BROOD INCUBATOR

The brood incubator (Fig. 4) may be made from an old box or cabinet. The one described here consists of a reconditioned pastry cabinet with a wooden back, glass sides, and a glass door; and measures approximately 21 inches wide, 21 inches deep, and 67 inches high. It contains three shelves, each having a three-fourths inch border cut away on all sides and an extension at each corner which

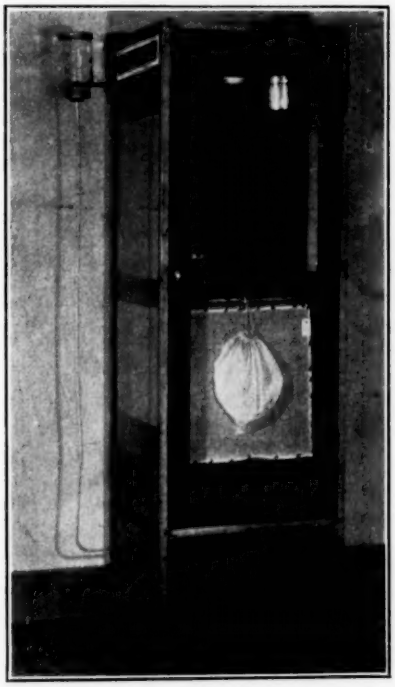

Fig. 4.—Brood incubator. The thermoregulator, which is supported by a galvanized iron arm, can be seen at the back. Arrangement of larval receptacles and fly cage is also shown. The water receptacle and tubing are shown at the left.

rests upon a support. This shape is used to facilitate a good circulation of air. On the undersurface of the bottom shelf is attached a sheet of insulating aluminum foil to prevent warping of the shelf by the heating unit below. The heating unit consists of two porcelain electric sockets, each containing a 125-watt electric light bulb. Humidity within the incubator is supplied by a shallow pan of water placed opposite the heating unit. If desired, humidity may easily be increased by immersing a heavy wad of cotton, cloth, sponge, etc., in the pan of water, or by suspending several strips of heavy absorbent cloth in the pan. Ventilation is maintained by several three-eighths inch holes bored in the lower right-hand and upper left-hand sides of the incubator. These are covered over with strips of screen which are fastened down with adhesive tape. Air enters

at the bottom and leaves at the top. The circulation can easily be regulated by plugging some of the holes with corks. This is done from the inside of the incubator.

Both the humidifier tray and heating unit are located on the floor of the incubator, the latter on the side where the holes for ventilation have been bored. At the top of the incubator a small section, with wooden sides and back, serves to hold larval receptacles. The fly cages are placed on the shelves below.

A convenient arrangement for bringing water into the humidifier tray is made by inverting a quart jar on a ring stand attached to the upper outer side of the incubator. The bottom of the jar, which now serves as the top, is cut away, so that water can be poured in, and is kept covered with a metal lid. Over the mouth of the jar is screwed a tin cover in which a hole is cut to receive a cork fitted with a glass tube. Attached to the glass tube is a rubber tubing at the end of which is a double right angle bend glass tube. A cork, fitted into a hole in the lower outer side of the incubator, holds this right angle tube in place. The water is kept from flowing out by a pinchcock.

4. THERMOREGULATOR

For regulating the temperature the device described by M. C. W.* was used with great satisfaction.† This thermoregulator (Figs. 5 and 6) consists of a three-inch ether-wafer unit,‡ originally intended for a poultry incubator. The wafer unit, which has a short threaded pin projecting from the center of the bottom surface, is fastended to the supporting bar (*a*) with a small nut (*b*). The wafer unit also has a hollow-end stud projecting from the center of the top surface, which receives the end of the adjusting rod (*c*). Two metal contact strips are adjusted on the base piece (*d*) so that one (*e*) is fastened to the adjusting rod by means of a small nut (*f*) which serves to support the adjusting rod (*c*). The other strip (*g*) is adjusted so that it makes contact with strip "*e*" at the proper point.§ The adjusting rod is threaded for some distance so that the temperature may be increased or decreased. When the wafer unit is warmed, the ether vapor inside expands and forces the hollow-end stud up against the end of the rod (*c*), causing the movable contact strip (*e*) to bend upward and break the contact. This turns off the heating unit. Loss of heat by circulation and diffusion through the sides of the incubator causes the temperature to drop, the wafer unit to contract, and the contact points to come together again. The lamps in the heating unit light; the disk expands and again breaks the circuit when the predetermined temperature has been reached. If it is desired to make the thermoregulator break the circuit at a higher temperature, the adjusting rod is turned in a counterclockwise direction. If a lower temperature is desired, the rod is turned

*M. C. W.: Inexpensive Incubator Cabinet for Microscope Cultures, Popular Science Monthly, 127: 86, S. M., 1935.

†The construction of the thermoregulator described here was modified somewhat by the author.

‡These come in different sizes and range in price from 35 to 60 cents. The size used will depend upon the dimensions of the incubator.

§Excellent contact strips suggested by M. C. W. are those which can be obtained by tearing apart an old telephone jack, which can be secured at a radio repair store or where miscellaneous radio and telephone equipment is available. These strips are already provided with contacts that produce a minimum of sparking.

in a clockwise direction. A helpful suggestion will not be out of place at this point. In some cases it was difficult to get the contact strips to touch exactly. For this reason, sensitivity was not very great. After some experimentation, however, it was found that by increasing the weight of the adjusting rod the strips could be made to touch each other and thus greater sensitivity could be

Fig. 5.—Thermoregulator. Note construction and arrangement of contact strips.

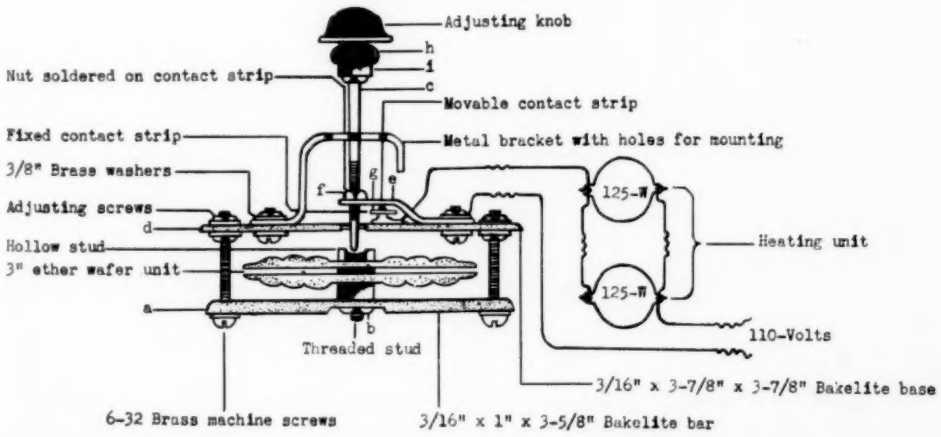

Fig. 6.—Diagram of thermoregulator showing construction of the mechanism.

effected. This was accomplished by fastening two lead washers (*h* and *i*) under the adjusting knob. The washers were made by pouring melted lead into a mold of the desired size. Holes were made in the washers by placing a greased metal rod in the center of the mold before pouring in the lead. With the aid of this adjustment the temperature was made to vary less than 0.4 of 1° C.

5. EGG COLLECTOR

Because it is not always possible to collect eggs soon after they are deposited, many of them are desiccated as a result of being exposed too long to the laboratory air, even in cases where the humidity is relatively high. The meat used to collect eggs becomes dry and leathery when exposed too long to the air and as a result, the feeding surface becomes extremely poor and the flies are less readily attracted to it. The device shown in Fig. 7 was found to be satisfactory in keeping both the meat and the eggs in good condition for longer periods of

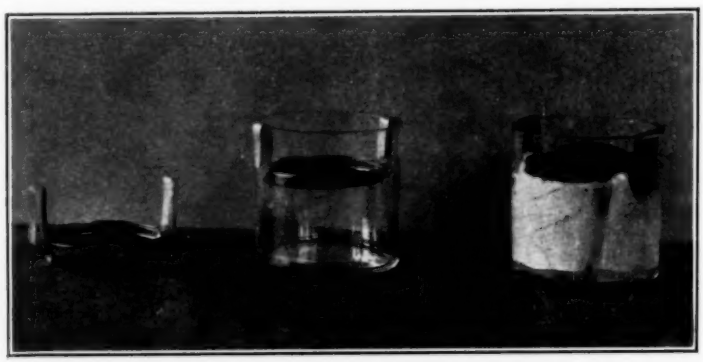

Fig. 7.—Egg collector. The meat is shown in the tray which is inside the stender dish. Note arrangement of cloth skirt.

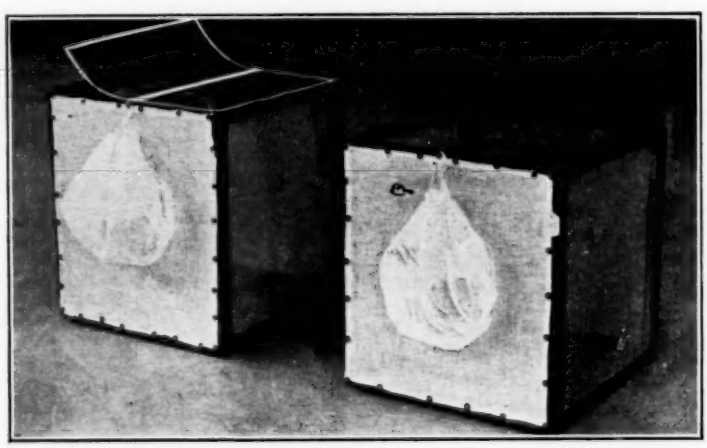

Fig. 8.—Fly cages. Note top arrangement in each cage. Both facilitate convenient and rapid entry into the cages.

time. This consists of a glass stender dish, measuring 100 by 100 mm., which is used as a water receptacle. In it, acting as a tray, is hung a metal jar cover which is slightly smaller than the diameter of the stender dish. Two pliable metal strips are screwed to the border of the jar cover and adjusted so that the cover can be placed at any desired height above the water. The stender dish is filled with water to a depth of two inches. A small absorbent cloth skirt is lapped over the tray which is then suspended in the stender dish about one inch above the surface of the water, with the cloth skirt hanging in the water. The skirt serves to draw up water by capillarity, to increase the surface of evaporation, and to

fill the space between the cover and the wall of the receptacle and so prevent flies from making their way to the water and drowning. On top of the cloth are placed several sheets of filter paper. On the filter paper is placed a small pat of ground, lean beef in which rather deep, obliquely directed pockets are made by pressing a finger into its lateral and upper surfaces. The meat is kept moist by the filter paper which draws up water from the cloth skirt. The flies appear to be quite fond of these little pockets and deposit their eggs in them. The pockets also serve to keep the eggs from being exposed too much to the air, and make it possible to leave eggs for longer periods of time without the danger of being desiccated.

6. FLY CAGE

The fly cage (Fig. 8) consists of a wooden frame and floor. It is covered on three sides with ordinary wire screen cloth and on top with isinglass. The front of the cage is covered with fairly heavy cheesecloth in the center of which is cut a circular hole about eight inches in diameter. Around the periphery of this hole is sewed a fourteen inch sleeve of the same material. At the free end of the sleeve a one-fourth inch hem is made. Two strings are drawn through this hem so that the mouth of the sleeve can be closed easily. The sleeve facilitates entry into the cage and prevents the escape of flies when entrance is necessary. The top of the cage is made to open in door fashion by attaching two small hinges to the back of the cage. Two small hooks are attached to the front of the cage so that the top can be locked. This arrangement affords quick access to the inside of the cage when cleaning is desired, and facilitates rapid removal of dead flies when the colonies are destroyed.

Another simple type of cover which allows convenient and rapid entry into the cage (Fig. 8) is made by placing a wooden bar across the center of the top of the cage and fastening to the bar, by means of thumbtacks, a bleached x-ray film or isinglass. A border of adhesive tape is placed around this cover to increase its weight as well as to obviate cutting by the raw edges. This arrangement has a spring effect and keeps the cover down tightly.

1410 N. W. TWENTY-FOURTH AVENUE

STUDIES ON THE KLINE TEST FOR SYPHILIS*

I. Technique of the Kline Test on Heated Serum, with Special Reference to the Quantitative Aspects

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INTRODUCTION

THE development of the various flocculation tests for syphilis has closely paralleled that of the complement fixation (or Wassermann) tests. Michaelis,[1] in 1907, was the first to observe that a precipitate appears when the serum of syphilitic patients is mixed with a saline extract of syphilitic liver. The antigen used by this investigator was similar to the one employed by Wassermann, Neisser, and Bruck[2] the year before in developing the Wassermann test. Marie and Levaditi,[3] and Landsteiner, Müller and Pötzl[4] found that extracts of tissues which did not contain the *Treponema pallidum* were satisfactory antigens for Wassermann tests, and the latter authors showed, furthermore, that alcoholic extracts of organs of normal animals made even more satisfactory antigens than the aqueous extracts of syphilitic organs which had previously been used. Yet, for some time thereafter Wassermann insisted on the use of syphilitic liver in preparing the extracts. Hecht[6] contributed to the subject by evolving an antigen which consisted essentially of an alcoholic extract of normal heart muscle, for use both for a Wassermann test and for a precipitation procedure. However, it was not until the introduction of Meinicke,[7] Sachs-Georgi,[8] Vernes,[9] Dreyer and Ward,[10] and Kahn[11] tests between the years 1918 and 1922 that the great possibilities of the flocculation method were demonstrated.

In the earlier stages of their development the chief advantage that the various flocculation tests had to offer was their simplicity; and their use was probably prompted by the fact that they were more economical with regard to reagents, glassware, other laboratory equipment and time. However, at the second laboratory conference of the League of Nations at Copenhagen in June, 1928, the better flocculation tests including the Kahn, Müller,[12] Sachs-Georgi, Dreyer and Ward, and Meinicke tests were found to be equal or superior to the better Wassermann tests, both in specificity and sensitivity. This conference resulted in a more widespread adoption of the flocculation tests, so that more and more laboratories began to use them in conjunction with or to the exclusion of the Wassermann tests.

All the better flocculation tests which had been developed up to ten years ago involved a macroscopic technique. In 1926, Kline and Young[13] demon-

*From the Department of Laboratories, Jewish Hospital of Brooklyn.
Received for publication, November 7, 1936.

strated that it was possible to use the Kahn antigen for a microscopic test carried out on glass slides without loss of sensitivity or specificity. It seemed, however, that just those properties of the Kahn antigen which made it superior to the other antigens in use for macroscopic precipitation tests, made its adaptation to a microscopic slide test difficult.

Most of the antigens in common use for precipitation tests are stable by virtue of the fact that they are relatively highly diluted and the individual particles in the opalescent antigen dilution are very small, many submicronic. Hence, precipitation tests done with these antigens are not very sensitive, and long periods of incubation up to twenty-four or even forty-eight hours are necessary. Kahn[11] shortened the period of incubation to only a few minutes, and at the same time increased the sensitivity of the test without diminishing its specificity, by using a concentrated *unstable* antigen. The Kahn antigen suspension contains coarse lipoidal particles which, however, disperse on coming in contact with additional saline solution or with serum. If the serum is derived from a syphilitic patient, a precipitate appears within a few minutes; if the serum comes from an individual free from syphilis, no precipitate results.

These very properties of high concentration and instability of the Kahn antigen emulsion carry with them the following disadvantages: (a) the majority of emulsions are unsatisfactory at low room temperatures; (b) the antigen emulsions do not yield reliable results unless they are used within an hour and a half.

By varying the method of extracting the antigen lipoid, and of mixing the ingredients which make up the antigen emulsion, Kline[13] succeeded in preparing a concentrated, yet *stable* antigen emulsion, which gives highly sensitive and specific results in a microscopic slide flocculation test. In fact, in a recent evaluation of serodiagnostic tests for syphilis, in the United States,[15, 16] in which Kline, Kahn, Hinton, Kolmer, Brem, Eagle, and others performed various modifications of flocculation and Wassermann tests, the Kline test proved equal or superior to the other tests, both in sensitivity and specificity.

During the past two years, the present author has performed, or has had performed under his supervision, over 5,000 Kline tests on heated serum, most of them in parallel with some modification of the Wassermann test. In addition, experiments and studies have been carried out on the various factors which influence the sensitivity and specificity of the reaction. The purpose of this and subsequent papers of this series is to present the results of these studies and practical experience with the Kline test. In order to emphasize certain special aspects of the procedure, the technique will be given in some detail, with the consent of the originator of the test.

PREPARATION OF REAGENTS FOR THE KLINE TEST

The Serum.—The patient's blood is most conveniently collected by venipuncture. After the blood is firmly clotted, the clot is rimmed and the serum separated by centrifuging. The serum is pipetted into a fresh tube with the aid of a capillary pipette and nipple. If a large number of serums are being separated, the same pipette may be used for all the serums, provided that the pipette is rinsed at least twice with normal salt solution before proceeding to the next specimen.

The tubes containing the serums are placed in a water-bath at 56° C. for thirty minutes, following which the serums are ready to be tested. This is done since fresh serums contain a thermolabile substance which interferes with the agglutination reaction, so that even serums containing a high titer of reagin may fail to produce a visible reaction if tested in the unheated state. Experiments conducted to determine the nature of this interfering substance, which in its behavior closely resembles complement, will be described in a later paper.

If venipuncture is to be avoided for any reason, an adequate amount of blood for a test can be obtained from a deep puncture of a finger in adults, or of the great toe in infants. In accordance with Rein's[17] suggestion, the author prefers to collect the blood in capillary tubes. These can be prepared from glass tubing, 4 mm. in diameter, with slight constrictions at 5½-inch intervals made by melting the glass in a Bunsen flame. The tubing is broken at the center of the constricted points with the aid of a small file. The blood is collected by alternately squeezing the finger until a drop of blood forms and touching the end of the capillary tube (which must be held slightly below the horizontal position) to the blood. When the tube is two-thirds full, the blood is allowed to run to the center of the vial, leaving about one inch at either side, and the ends of the tube are sealed in a Bunsen flame. Blood collected in this way can be sent through the mails, and Rein has examined many such blood specimens received from various parts of the country for the Metropolitan Insurance Company.*

Enough serum can be obtained from one capillary tube of blood for three Kline tests. By taking two vials of blood it is possible to do a quantitative test on positive specimens. To separate the serum, one end of the vial is opened with a file and a wire inserted to break up the clot. The vial is then centrifuged for five minutes at high speed, and the supernatant serum pipetted off into a small clean tube with the aid of a fine capillary pipette. After being heated at 56° C. for half an hour, the serum is ready to be tested.

The Antigen Lipoid.—For the preparation of the emulsion used for the Kline test, the following ingredients are necessary: an alcoholic solution of purified beef-heart lipoids, an alcoholic solution of pure cholesterol, physiologic saline solution and distilled water. The preparation of the antigen lipoid from beef heart offers the greatest technical difficulty that Kline's test has to offer.

In many of the flocculation tests and Wassermann tests in use today, simple alcoholic extracts of normal organs are still being used for the antigen emulsions. By 1911, however, Noguchi, and Noguchi and Bronfenbrenner[18] had found that when simple alcoholic extracts of beef heart were added to acetone, a brown waxy substance was precipitated which contained the most potent and specific antigenic substances of the original extract. The acetone soluble fraction, on the other hand, increased the anticomplementary and hemolytic action of the extract and at the same time was responsible for occasional false positive Wassermann reactions. The antigen used for the Kahn test contains a small amount of acetone soluble and ether insoluble, alcohol soluble impurities, and Kline found that the removal of these impurities resulted in an antigen which was highly satisfactory for use in his microscopic flocculation test. The method finally evolved by Kline for obtaining a purified extract of beef heart phosphatids is described in his book,[14] and will not be repeated here.

During the past three years, we have had occasion to prepare three lots of antigen lipoid in accordance with Kline's directions.† On two occasions we obtained antigen extracts which were highly satisfactory, since they gave reactions identical both qualitatively and quantitatively with those obtained when using antigen which B. S. Kline was kind enough to give us. One lot of antigen, on the other hand, evidently contained some impurities, since it frequently gave faint reactions with serum from nonsyphilitic patients. When the nature of these faint reactions was recognized, and when such weak reac-

*This technique can also be used for collecting blood specimens for other serologic tests, such as blood grouping and Widal tests.

†These were prepared by Miss Elizabeth Eckert, technician in the Division of Serology.

tions were disregarded, it was possible to obtain results equal both in specificity and sensitivity to those obtained with the pure antigen. However, for best results, it is essential to use the purest antigen extract possible.

Lvoff[19] studied lots of Kline antigen which he had kept for periods of two, nine, eleven, and fourteen months, in comparison with fresh antigen, and believed that he detected a slightly increased sensitivity for weak positives with age. He concluded that it was still safe to use the antigen after six months. In our experience, the antigen extract once prepared seems to last indefinitely. Thus far, we have only been able to study this phase for periods up to eighteen months, but during that interval, we could not detect any change in antigen lipoid which had been kept in hermetically sealed vials at room temperature. Kline[14] remarks that when kept in small glass-stoppered bottles which are opened frequently, the antigen shows no appreciable change for at least six months, but for safety, he places an expiration date of six months on lots of antigen prepared in his laboratory. Even under these conditions, however, the present author has found the antigen safe to use for periods well over a year, provided that adequate controls are included in all tests so that deterioration can be detected as soon as it sets in.

In certain Wassermann and flocculation tests, extracts of horse heart or of human heart are used in place of extracts of ox heart for preparing the antigen. Undoubtedly, extracts from these sources could also be used for the Kline test. However, while the organs of the ox do not contain Forssman antigens, these antigens are present in the horse. Hence, the use of extracts prepared from the horse heart would be more likely to give false positive reactions in diseases in which heterophile antibodies appear, such as pneumococcus infections, infectious mononucleosis, etc., and even in certain normal individuals with a sufficient titer of natural heterophile antibody.*

The Cholesterol Solution.—The cholesterol solution used for making the Kline antigen emulsions is prepared by placing 1 gm. of crystallized cholesterol (Pfanstiehl) in a 100 c.c. volumetric flask, and filling the flask to the mark with absolute alcohol. This is placed in an air incubator at 37° C. and the cholesterol goes into solution overnight. Once prepared, the solution must be kept in the incubator constantly, only being removed when needed. Not infrequently it is found, usually after a period of several months, that the solution has undergone a sudden change which renders it unfit for further use.

Cholesterol plays an essential part in the Kline test by its effect on the dispersion of the antigen lipoid. The fact that a much higher titer of antibodies is required for precipitation than for agglutination tests has been observed since studies in immunology were first inaugurated. In this regard, Zinsser[21] points out that this phenomenon can be traced to the fact that the units in agglutination tests are much more coarsely dispersed than those in precipitation tests, so that there is a much smaller total surface, and less antibody is required to cover the surface and produce a positive reaction. This principle has been made use of to increase the sensitivity of flocculation tests for syphilis. Thus, Sachs and Rondoni[22] showed that emulsions prepared from alcoholic extracts, by slowly mixing with saline, are more coarsely dispersed and more sensitive than emulsions made by rapidly mixing the ingredients.

*Cf. Taniguchi.[20]

Similarly, Kline points out that the slow addition of his antigen lipoid to a minimal amount of water or saline solution results in a suspension of coarse globular particles (average diameter about 6 micra), which is readily flocculated by strongly positive syphilitic serums. And, as has already been pointed out, a similar principle is made use of in the Kahn test. A better method of converting a precipitation procedure into an agglutination test is by coating the specific substances on suitable particles in suspension. Thus, Jones[23] reports that collodion particles coated with proteins are agglutinated by high dilutions of the homologous precipitins, and Landsteiner and van der Scheer[24] showed that blood corpuscles coated with the soluble specific substances from cholera vibrio are agglutinated by high dilutions of precipitating anticholera immune serums. Various substances have been employed in a similar way to increase the sensitivity of the complement fixation and flocculation tests for syphilis, such as cholesterol, Balsam of Tolu, sitosterol, and other materials with similar physical properties. Of these, cholesterol is the most widely used and this substance also helps to increase the sensitivity of the Kline test.

As early as 1910, Browning, Cruikshank and M'Kenzie[25] showed that the highly specific acetone insoluble fraction of alcoholic extract of beef heart is less sensitive in Wassermann tests than the crude alcoholic extract, but when cholesterol is added, the sensitivity is increased up to and beyond that of the crude extract. At the present time, in many Wassermann tests and flocculation tests for syphilis, cholesterol is added to increase the sensitivity of the reactions. When preparing the emulsions for most flocculation tests, the usual procedure is merely to dilute the cholesterinized antigen extract with saline or distilled water. The particles of such an emulsion consist of a fairly uniform mixture of cholesterol, antigen lipoid, and adventitious substances. Furthermore, the particles are not uniform in size or shape and are affected by such factors as the amount of water present, the temperature, and the speed with which the ingredients are mixed. The emulsions used for the Kline test, on the other hand, are prepared by first precipitating the cholesterol crystals in water and then coating them with pure antigen lipoid. In this way it is possible to obtain stable, concentrated emulsions of uniform platelike crystals of cholesterol coated with the specific antigen lipoid. The Kline test owes its high sensitivity to the fact that it is an agglutination rather than a precipitation test, and the Kline antigen suspension is entirely comparable to blood cell or bacterial suspensions, except that the individual particles in the former consist of cholesterol crystals coated with beef-heart lipoid instead of red blood cells or bacteria. (According to Kline, the average size of the particles in his emulsion is about 5 by 3 by 1 micra.)

The Saline Solution.—The saline solution used in the Kline test is physiologic (isotonic with blood). It is most advisable to prepare a stock 10 per cent solution from which enough normal saline solution for the day's needs can be prepared by diluting 8.5 c.c. quantities to 100 c.c. with distilled water.

Preparation of the Antigen Emulsion.—Kline gives methods for preparing various antigen emulsions depending whether it is intended to test heated or unheated serum, defibrinated blood, or spinal fluid. In the present paper we shall deal only with the antigen emulsions used in tests on heated serum.

First, a basic emulsion is prepared as follows: Into a one-ounce bottle, 0.85 c.c. of distilled water is measured. To this is added 1 c.c. of the 1 per cent cholesterol solution and the bottle is then held at the neck and gently rotated for twenty seconds. One-tenth cubic centimeter of antigen lipoid is allowed to run into the mixture by pipetting it along the inside of the neck of the bottle. The bottle is corked and the mixture is emulsified by vigorously shaking it back and forth for one minute by hitting the bottom of the bottle against the palm of the hand rapidly. Then 2.45 c.c. of normal saline solution are added and the emulsion shaken for another minute, but not as vigorously as before. (The distilled water, saline and cholesterol are measured out with 1 c.c. pipettes graduated in tenths; the antigen lipoid with a 0.2 c.c. pipette graduated in hundredths.) The mixture which results is the "raw" emulsion and is not ready for use before it has been treated further.

The "raw" emulsion could be used, if desired, for testing purposes. However, it will be found that if the raw emulsion is allowed to stand at room temperature it gradually increases in sensitivity. Only after a variable period of many hours does it finally reach a constant level of sensitivity. This property of the raw emulsion makes it undesirable for use for diagnostic tests. The process of ripening of the emulsion can be hastened in several ways. Two methods in particular were developed by Kline: one method is used to prepare his so-called diagnostic emulsion; with the other method an emulsion for an exclusion test is obtained.

The reagent for the diagnostic test is prepared from the raw emulsion by placing the latter in a water-bath at 35° C. for fifteen minutes. (Placing the test tube of the emulsion in a glass of warm water in the laboratory air incubator at 37° C. will serve the purpose). The reagent is then ready for use.

The exclusion emulsion is prepared by incubating 2 c.c. of the raw emulsion in a water-bath at 56° C. for fifteen minutes. This is then poured into a 3 by 1 inch tube and centrifuged at 2,000 revolutions per minute for fifteen minutes. The supernatant fluid is decanted and while the tube is kept inverted, its inside is wiped with a clean cloth up to the level of the sediment. Then 1.5 c.c. of normal saline solution are added and the reagent is ready for use.

THE TESTS

Qualitative Procedure.—Once the reagents have been properly prepared, the test itself is simple. In principle, just as for a blood grouping or Widal test, all that is required is to mix the patient's serum with the antigen emulsion, and then to examine the mixture for clumping under the low power of the microscope. If the serum comes from a syphilitic patient, the platelike particles will clump together into large masses visible to the naked eye, except with serum containing the smallest amounts of reagin, whereas serum from a patient free from syphilis or diseases such as yaws, leprosy, and malaria, etc., will have no apparent effect on the suspension, even when examined under the microscope. The appearance of the reactions in the Kline test in every way resembles that of an agglutination reaction except that crystals have been substituted for the red blood cells or bacteria.

For the tests, Kline used special glass slides* (2 by 3 inches) on each of which twelve paraffin rings (inside diameter 14 mm.) have been made. (For the method of preparing these paraffin ring slides see Kline's book.) The capacity of each of the wells formed by the paraffin rings is slightly over 0.05 c.c. In addition, Kline describes a method of preparing a special tray (from covers of slide boxes) which will hold three of the paraffin ring slides. Finally, a special holder for two trays can be used, making it possible to do as

*These slides are also useful for other immunological tests, such as blood grouping tests.

many as 72 tests at one time. To do a greater number of tests simultaneously is not practicable, since then the factor of evaporation would affect the reactions.

The actual tests are carried out as follows: 0.05 c.c. of each of the serums to be tested is pipetted into corresponding paraffin ring wells with the aid of 1 c.c. graduated pipettes. Then, with the aid of a fine Wright capsule, approximately 0.008 c.c. of antigen emulsion is added to each well. The slides are rapidly rotated on a flat surface (about 4 complete circular movements per second) for four minutes, after which time the reactions are read under the low power of the microscope.

The present author prefers using a capillary pipette and nipple for measuring out the patient's serum to the use of a graduated pipette. It is a simple matter to draw out a pipette from glass tubing which will deliver approximately 20 drops per c.c., and one drop of serum from such a pipette is used for the test. A single pipette can be used for all the serums tested, provided that the pipette is rinsed twice with normal saline solution after each serum. For a description of the Wright pipette used for measuring out the antigen emulsion see Kline's book. These pipettes are drawn out from glass tubing and are of such caliber that they deliver approximately 60 drops for each 0.5 c.c. of fluid.

If only a few serums are to be tested, the diagnostic and exclusion tests can be done simultaneously, preferably on separate slides. When many serums are being examined, the two tests should be performed separately, so that one test can serve as a check on the other, and clerical errors can be prevented.

The strengths of the reactions are graded according to the size of the clumps from one-plus (+) up to four-plus (++++). In the negative tests, the antigen emulsion is totally unaffected, and each particle remains separate and distinct. In the stronger reactions (+++ and ++++), the clumps are so large as to be visible to the naked eye. The disadvantage of this method of scoring is that while it classifies reactions weaker than four-plus, it does not differentiate reactions stronger than four-plus, and one cannot tell how high the titer of the reagin is. For that reason, the present author has made it a practice to titrate all positive serums and report the results in terms of reagin units.

Quantitative Procedure.—The method of determining the number of reagin units present in a syphilitic serum is to prepare a series of progressively halved dilutions of the serum, and then test each dilution separately in order to determine the highest dilution which still gives a distinct positive reaction (at least two-plus). The sample protocol given in Table I illustrates titrations against the emulsion for the Kline exclusion test with a number of selected serums.

Table I exhibits certain interesting features. One is immediately impressed with the fact that a four-plus reaction may represent anything from 4 reagin units to a thousand (the most potent serum the present author has thus far encountered contained slightly over 1,000 reagin units against the exclusion antigen). In addition, occasional serums are encountered containing high titers of reagin which do not react as strongly on the direct test as serums

containing less reagin. Thus, serum 103D, which contains 128 reagin units only, gave a three-plus reaction on the direct test, whereas serum 12D, which only contained 2 reagin units, gave a somewhat stronger reaction. Due to the occurrence of this slight prozone with certain strongly positive serums, the titration of all positive serums is advisable. Finally, the author has encountered more than ten serums thus far which react like serum 134D (in Table I).

TABLE I

QUANTITATIVE KLINE TEST
(PERFORMED WITH ANTIGEN FOR EXCLUSION TEST)

SPECIMEN	SERUM DILUTION									NUMBER OF REAGIN UNITS
	UNDILUTED (1:1)	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
2D	++++	++++	++++	++±	-	-				8
3D	++++	++++	++++	+++±	++	tr.	-			16
9D	++++s*	++++s	++++s	++++	++++	+	-			16-32
12D	+++±	++±	+	-	-					2+
75D	+++	±	-							1
96D	-	-	-							0
100D	++++	++++	+++	-						4
103D	+++	++++	++++	++++s	++++s	++++	++++	+++	-	128
104D	+±	-								less than 1
105D	±	-								0
134D	tr.	±	+±	++	++±	tr.	-			0

*++++s (four-plus strong) represents a very marked reaction with large macroscopic clumps which are so few in number that they can almost be counted.

Serums of this sort give a weak reaction on the direct test which becomes more distinct, though never very strong, with the diluted serum. This phenomenon is not due to specific reagin and is not diagnostic of syphilis. Investigations are under way to determine its nature and cause.

A rapid and convenient method of titrating positive serums, which is used by the present author, is as follows: A series of small tubes (inside diameter 8 mm.) is set up, and to each tube the same number of drops (usually 5 drops) of saline solution is added. One drop (0.05 c.c.) of the undiluted serum is placed in the first well of a paraffin ring slide, and then the proper number of drops, 5 drops, if 5 drops of saline were used, of serum is placed in the first of the series of tubes, the remainder of the serum being returned to the original tube. The serum and saline are thoroughly mixed by drawing up and down in the pipette several times and one drop (0.05 c.c.) of the 1:2 dilution of the serum is placed in the second well of the slide. At the same time 5 drops are transferred to the second tube in the series, and the remainder of the diluted serum returned to the first tube or discarded. By mixing the saline and 1:2 dilution of serum in the second tube, a 1:4 dilution is prepared and the process is repeated carrying out the titration as far as is desired. The pipette is then rinsed thoroughly with saline, and one can proceed with the next positive serum. After all the dilutions have been set up, a small drop (0.008 c.c.) of antigen emulsion is added to each well, and the slides are rapidly rotated on a flat surface for four minutes, after which time the reactions are read.

The quantitative technique makes it possible to analyze more thoroughly the various factors which affect the sensitivity and specificity of the Kline test,

and the subsequent papers of this series will deal with questions of that nature. For example, one immediately discovers that the Kline exclusion emulsion is approximately twice as sensitive as the Kline diagnostic test emulsion (see Table II). With occasional serums, the exclusion units may equal 4 times the diagnostic units, and, on the other hand, with rare serums the titer is the same against both antigen emulsions.

TABLE II

RELATIVE SENSITIVITY OF KLINE DIAGNOSTIC AND EXCLUSION TEST EMULSIONS

SERUM NO.	EMULSION USED	DILUTION OF SERUM								NUMBER OF REAGIN UNITS
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	
1	Diagnostic	++++s	++++s	++++s	++++	+++±	+++	+	−	32+
	Exclusion	++++s	++++s	++++s	++++s	++++	+++±	+++	+	64+
2	Diagnostic	+++	+±	−	−					2-
	Exclusion	++++	++±	++	−	−				4
3	Diagnostic	±	−	−	−					0
	Exclusion	++±	±	±	−	−				1
4	Diagnostic	++++	+++±	±	−	−				2
	Exclusion	++++	+++±	++±	+	−	−	−		4
5	Diagnostic	++±	−	−	−					1
	Exclusion	+++±	+++	+±	−	−				4-

STANDARDIZATION OF THE KLINE TEST

As has already been mentioned, the Kline test proved equal or better than a series of other flocculation and Wassermann tests in an evaluation of serologic tests for syphilis[15, 16] carried out by the U. S. Public Health Service in 1935. However, the tests in this evaluation were carried out by serologists who originated the respective methods compared. A test which gives good or even excellent results in the hands of the originator is of no practical value if these results cannot readily be duplicated in other laboratories. The U. S. Public Health Service has just completed a second evaluation[27] in which this aspect was studied, namely, how results obtained in laboratories distributed throughout the country compare with those of the originators of the methods in question. By participating in this evaluation, the present author had the unique opportunity of comparing the Kline tests performed in his own private laboratory with tests carried out on the same blood specimens in the laboratories of Kline and Rein. A summary of these results is given in Table III. It will be seen that except for the fact that the exclusion antigen used by the author was slightly too sensitive, our results compare very well with those of Kline and Rein.

In reporting the reactions to the U. S. Public Health Service, each serum had to be classed as negative, doubtful, or positive. The system of scoring adopted by the present author was to consider as negative those serums which gave no or only a ± reaction; serums giving a + to ++ reaction on the direct test were classed as doubtful—such serums no longer gave any reaction when diluted; and finally serums containing between 1 and 1,000 reagin units were called positive. The slight discrepancy between our results and those of Kline and Rein can be traced to that narrow zone between serums giving doubtful

reactions and serums containing only 1 or 2 reagin units. When the actual protocols of Dr. Rein and the present author were compared upon completion of the evaluation, it was found that the quantitative results, as judged by the intensity of the reactions on the direct test, exhibited an almost perfect agreement. This is all the more remarkable since the tests were performed by technicians with entirely different training, and in addition, entirely different reagents, prepared in the respective laboratories, were used.

The process of standardizing the reagents for the Kline test is extremely simple in comparison to the procedure which must be followed for the Wassermann tests. Once the antigen lipoid has been prepared, all that is required is to prepare antigen emulsions and perform tests on a short series of positive and negative serums in parallel with emulsions prepared from a standard antigen lipoid. The process of standardization can also be carried out with only a single positive and negative serum by titrating the serums in parallel against the new and the standard antigen emulsions. The only difficult part of the procedure is in preparing the antigen lipoid, but the laboratory technician can even be spared that work since standard antigen lipoid for Kline tests can be obtained in the open market.

TABLE III

DEMONSTRATING STANDARD CHARACTER OF KLINE TEST WHEN PERFORMED BY INDEPENDENT INVESTIGATORS

TYPE OF TEST DONE	SOURCE OF BLOOD SPECIMENS	NUMBER OF SPECIMENS MAILED	INVESTIGATOR	NUMBER OF SPECIMENS TESTED*	PERCENTAGE OF POSITIVE REACTIONS
Kline Diagnostic Test	Syphilitic donors	214	Wiener	208	89.4†
			Kline	206	88.8
	Normal donors	103	Wiener	102	0.0
			Kline	103	0.97
Kline Exclusion Test	Syphilitic donors	232	Wiener	231	87.0†
			Rein	230	84.8
	Normal donors	104	Wiener	104	3.03
			Rein	102	0.0

*These figures are slightly lower than the number of specimens sent on account of breakage en route, etc.

†The blood specimens tested in these two series came from different sets of patients. If the blood had come from the same patients the percentage of positive reactions would naturally have been higher for the exclusion test.

Every antigen emulsion prepared from a standard antigen lipoid gives identical results so that it is relatively easy to maintain a constant level of specificity and sensitivity from day to day, and from month to month, as long as the supply of antigen lipoid lasts. To maintain a consistent level of sensitivity and specificity with a Wassermann test is a much more difficult task. The reason for this is that the process of standardization is not completed when the antigen has been titrated, since each day that tests are done, it is necessary to titrate the guinea pig complement, sheep cell amboceptor and standardize the strength of the sheep cell emulsion. Even when the preliminary titration seems satisfactory, it not infrequently happens in the small laboratory that all the tests fail to hemolyze, or that many of the serums are "anticomplementary."

The New York State Department of Health has carried its Wassermann procedure to a high state of perfection, but only by systematizing the work like the various steps of a manufacturing process. The expense of duplicating its

procedure in a small laboratory would be prohibitive. In this connection Irving Derby,[26] Pathologist at the Brooklyn State Hospital, has been performing Kline tests on a series of blood specimens which are also tested at the New York State Department of Health Laboratories. He has found, in a series of well over 3,000 specimens tested to date, that the Kline test gives 4 to 5 per cent more positive reactions in syphilitic individuals than the New York State Wassermann, yet there is no significant difference in the number of false reactions in nonsyphilitic individuals. Derby performs his tests single-handed with a minimum of equipment in only two or three hours, while the Wassermann tests on the same specimens take several technicians a full day to perform.

Additional advantages of the Kline test are: Specimens which are anticomplementary in the Wassermann test can be examined by Kline's test, since even deep hemolysis or jaundice does not interfere with the reaction. If there is a precipitate in the serum, this may be removed by centrifuging, and the supernatant serum can be used for the test. Even if the turbidity cannot be cleared by centrifuging, it is still possible to perform a Kline test, since with experience one can recognize the unclumped crystals of the negative reaction and the clumped crystals of the positive reaction under the microscope, even in the presence of extraneous débris. On the other hand, the presence of precipitates in a serum would interfere with the reading of macroscopic flocculation tests such as the Kahn test.

The remarkable stability of the Kline diagnostic and exclusion test emulsions virtually makes them stock reagents, so that the Kline test is convenient where it is necessary to perform a serologic examination quickly, as on blood donors before a blood transfusion. Kline states that the emulsions are safe to use for at least forty-eight hours. In the experience of the present author, the emulsions are suitable for even longer periods, often up to ten days or more. The only precaution necessary is to include at least one positive and one negative control; if these serums react satisfactorily, one can be certain that the remainder of the reactions are reliable.

In conclusion, it must be mentioned that despite its simplicity, the Kline test, like any other test, has its pitfalls and sources of error with which one becomes thoroughly acquainted only after long experience. Naturally, the technician who performs the tests must be properly trained, but the period of training required is shorter than that needed to learn the Wassermann test.

SUMMARY AND CONCLUSION

The Kline test is a microscopic agglutination test, which from the technical standpoint is in every way analogous to a blood grouping or bacterial agglutination test. The antigen lipoid used is a purified, acetone-insoluble, alcoholic extract of beef heart. The antigen emulsions consist of suspensions of uniform crystals of cholesterol, coated with antigen lipoid. The technique of preparing the test emulsions and performing the tests is described in detail.

The Kline test is equal or superior, both in sensitivity and specificity, to the better Wassermann and flocculation tests in general use today. The procedure is easy to standardize and maintain at a constant level of sensitivity.

Thus, in parallel tests performed on a series of more than 600 blood specimens, the present authors, Kline and Rein, obtained almost identical results, despite the fact that the tests were performed under entirely different laboratory conditions and with entirely different reagents. Another advantage of the test is the remarkable stability of the antigen emulsion, so that the emulsions can be used as stock reagents, particularly for tests on blood donors before transfusions. Finally, with the Kline test one can obtain optimum results as far as specificity and sensitivity are concerned, with the expenditure of a minimum amount of effort, time, and money for reagents and equipment.

A simple method of performing a quantitative Kline test is described, and the value of performing quantitative tests on all serums giving positive reactions is indicated. Subsequent papers will describe the use of the quantitative procedure in experimental studies on the Kline test.

REFERENCES

1. Michaelis, L.: Präcipitinreaktion bei Syphilis, Berl. klin. Wchnschr. **44**: 1477, 1907.
2. v. Wassermann, A., Neisser, A., and Bruck, C.: Eine serodiagnostische Reaktion bei Syphilis, Deutsch. med. Wchnschr. **32**: 745, 1906.
3. Marie, A., and Levaditi, C.: Les "Anticorps Syphilitiques" dans le liquide céphalorachidien des paralytiques généraux, Ann. d' l'Inst. Pasteur **21**: 138, 1907.
4. Landsteiner, K., Müller, R., and Pötzl, O.: Zur Frage der Komplementbindungsreaktion bei Syphilis, Wien. klin. Wchnschr. **20**: 1565, 1907.
5. Bruck, C., and Hidaka, S.: Ueber Fallungserscheinungen beim Vermischen von Syphilisseren mit alcoholischen Luesleberextrakten, Ztschr. f. Immunitätsforsch. u. exper. Therap. **8**: 476, 1911.
6. Hecht, H.: Zum Wesen der Wassermannschen Reaktion, Prag. med. Wchnschr. **39**: 316, 1914.
7. Meinicke, E.: Zur Methodik der serologischen Luesdiagnostik, München. med. Wchnschr. **65**: 1379, 1918.
8. Sachs, H., and Georgi, W.: Zur Serodiagnostik der Syphilis mittels Ausflockung durch cholesterinierte Extrakte, Med. Klin. **14**: 805, 1918.
9. Vernes, A.: Sur la precipitation d'un colloid organique par le serum humain, normal, ou syphilitique, Compt. rend. Acad. d. sc. **166**: 575, 1918.
10. Dreyer, G., and Ward, H. K.: A Simple Quantitative Serum-Reaction for the Diagnosis of Syphilis and the Expression of the Results in Standard Units, Lancet **1**: 956, 1921.
11. Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation, Baltimore, 1925, Williams and Wilkins Co.
12. Müller, R.: Ballungsreaktion (Conglobation) mit luetischen Seris, Arch. f. Dermat. u. Syph. **151**: 231, 1926.
13. Kline, B. S., and Young, A. M.: A Microscopic Slide Precipitation Test for Syphilis—Preliminary Report, J. A. M. A. **86**: 928, 1926.
    A Microscopic Slide Precipitation Test for Syphilis (Second Communication), J. Lab. & Clin. Med. **12**: 477, 1927.
14. Kline, B. S.: Microscopic Slide Precipitation Tests for Syphilis, Baltimore, 1932, Williams and Wilkins Co.
15. Cumming, H. S., Hazen, H., Sanford, A. H., Senear, F. E., Simpson, W. M., and Vonderlehr, R. A.: The Evaluation of Serodiagnostic Tests for Syphilis in the United States, J. A. M. A. **104**: 2083, 1935.
    The Evaluation of Serodiagnostic Tests for Syphilis in the United States, Supplement No. 1 to Venereal Disease Information, 1935.
16. Rein, C. R.: The Value of the Kline Exclusion in the Serodiagnosis of Syphilis, Am. J. Syph., Gonor. & Ven. Dis. **20**: 515, 1936.
17. Rein, C. R., and Le Moine, M.: Value of the Kline Test for Detection of Syphilis in Applicants for Insurance, Arch. Dermat. & Syph. **30**: 190, 1934.
18. Noguchi, H., and Bronfenbrenner, J.: Biochemical Studies on So-Called Syphilis Antigen, J. Exper. Med. **13**: 43, 1911.
19. Lvoff, R. F.: The Effect of Ageing on Kline Antigen, Chinese Med. J. **49**: 236, 1935.
20. Taniguchi, T.: A Source of Fallacy in the Wassermann Reaction Depending on Heterogenetic Antibody in Human Serum, J. Path. & Bact. **23**: 368, 1919.

21. Zinsser, H.: Note on the Quantitative Relations of Antigen and Antibody in Agglutination and Precipitation Reactions, J. Immunol. **18**: 483, 1930.
22. Sachs, H., and Rondoni, P.: Beiträge zur Theorie der Wassermannschen Syphilisreaktion. Ueber den Ersatz der Organextrakte bei der Reaktion, Ztschr. f. Immunitätsforsch. u. exper. Therap. **1**: 132, 1909.
23. Jones, J.: Agglutination by Precipitin, J. Exper. Med. **48**: 183, 1928.
24. Landsteiner, K., and van der Scheer, J.: Quoted in Landsteiner, K.: Specificity of Serological Reactions, Springfield, 1936, Charles C. Thomas, p. 45.
25. Browning, C. H., Cruickshank, J., and M'Kenzie, I.: Constituents Concerned in the Wassermann Syphilis Reaction, With Special Reference to Lecithin and Cholesterol, J. Path. & Bact. **14**: 484, 1910.
26. Derby, I. M.: Personal communication.
27. Parran, T., et al.: Efficiency of State and Local Laboratories in the Performance of Serodiagnostic Tests for Syphilis, Am. J. Clin. Path. **7**: 20, 1937; Ven. Dis. Inform. **18**: 4, 1937.

DETERMINATION OF IODINE IN BIOLOGICAL MATERIALS*

A Simplified Technique

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LEIPERT (1933, 1934) proposed a method of iodine determination which was modified by Trevorrow and Fashena (1935, 1936). The procedure here described is based on these methods but has been simplified to require less time, reagents, and apparatus. Hundreds of duplicate analyses have been run on blood, urine, feces, food, etc., with satisfactory results. Clinical data will be reported elsewhere.

REAGENTS

Iodine-free water. Distilled water is redistilled from potassium hydroxide solution. It is used in preparing all aqueous solutions below.

Sulphuric acid: Grasselli's C.P. sulphuric acid and Baker's C.P. sulphuric acid low in nitrogen and arsenic have both been satisfactory.

Three per cent sulphuric acid: 50 c.c. of concentrated sulphuric acid are boiled thirty minutes with 2 drops of 30 per cent hydrogen peroxide. The 3 per cent aqueous solution is prepared from this.

Five per cent ceric sulphate: A 5 per cent solution of G. Frederick Smith's anhydrous ceric sulphate is made in 3 per cent sulphuric acid.

Chromic acid solution: 250 gm. of chromic acid are dissolved in 150 c.c. of water, the solution containing about 1 gm. of acid per c.c. Schering-Kahlbaum chromic acid low in sulphate contained the least iodine of any samples analyzed.† Some lots of Coleman and Bell's C.P. grade and some lots of Merck's C.P. grade low in sulphate have been satisfactory.

Fifty per cent phosphorous acid: Schering-Kahlbaum anhydrous grade is dissolved to make a 50 per cent aqueous solution. Baker's 50 per cent aqueous solution has also been satisfactory if purified by steam distilling through it an equal volume of water.

One molar aqueous potassium hydroxide: Schering-Kahlbaum best grade potassium hydroxide in sticks has been satisfactory.

0.0007 molar (0.02 per cent) aqueous methyl orange.

*From the DeCourcy Clinic.
Received for publication, November 12, 1936.

†The Mutual Chemical Co. of Baltimore, Md., has more recently supplied us with many pounds of chromic acid very low in iodine content and reasonably priced.

Bromine: Schering-Kahlbaum best grade has been satisfactory. The bromine should be carefully tested for iodine as it is a common impurity.

One per cent aqueous potassium iodide: This is prepared from iodate-free iodide every two weeks and is kept in the dark in a refrigerator.

One per cent aqueous starch: This is prepared every two weeks from Merck's soluble starch by suspending the starch in cold water, adding the suspension to boiling water and boiling one minute. The solution is kept in a refrigerator.

0.0005 N sodium thiosulphate: This is prepared weekly by diluting 0.50 c.c. of a stock solution of 0.1 N sodium thiosulphate to 100 c.c. Both solutions are kept in the dark in a refrigerator. The 0.1 N solution is checked three times a year against standard potassium iodate solution. Its strength has remained constant for over a year.

Carborundum pieces (No. 6 mesh) are boiled ten minutes in dilute nitric acid, boiled ten minutes in dilute phosphorous acid, rinsed, boiled several times in iodine-free water and heated to redness for five minutes.

Potassium oxalate was found to contain iodine. Before using as anticoagulant, it is recrystallized by a single precipitation from saturated aqueous solution with ethanol.

A blank analysis of these reagents should not give a figure over 0.25 gamma of iodine for the quantities used in analyzing 10 c.c. of blood. Higher results have been traced to iodine in the chromic acid for the most part, but also to iodine in the bromine and in the potassium hydroxide.

APPARATUS

A Pyrex glass apparatus with interchangeable ground glass joints* is used. It consists of a 500 c.c. digestion flask, a 10 c.c. funnel, a connecting arm with trap,† and a condenser. The receiver is a 100 c.c. Pyrex extraction flask. Connecting arms without traps and with or without glass wool plugs in them, and arms with Kjeldahl connecting bulbs were both unsatisfactory with this type of apparatus. This size digestion flask has been satisfactory for analyses on smaller amounts of material than 10 c.c. of blood. A Bunsen burner is used for heating the digestion flask and a micro burner for heating the receiver.

PROCEDURE

Ten cubic centimeters of blood are pipetted into the digestion flask and the inside of the neck of the flask rinsed down with 10 c.c. of iodine-free water. A piece of carborundum is added. Seventeen cubic centimeters of chromic acid solution are added and quickly followed (caution), with cooling under the tap, by 100 c.c. of sulphuric acid added at first in portions of a few cubic centimeters but more rapidly after much chromic acid has precipitated, the digest meanwhile being stirred by shaking the flask between additions. The flask is placed on its stand in the fume hood, 1 c.c. of ceric sulphate solution is added, and heating is begun immediately at a moderate rate. The addition of reagents takes about three minutes. Speed is essential in these steps if iodine is not to be lost. Heating is continued until bubbling due to decomposition of the excess chromic acid has almost ceased and on addition of a few cubic centimeters of water, white fumes fill the flask. This takes about ten minutes. Bumping occurs if the digest becomes too hot (over 210° C. usually) and chromous sulphate precipitates.

*The Scientific Glass Apparatus Co., Bloomfield, N. J., made this apparatus.
†Designed by Mr. Normal L. Matthews, Columbus, O.

When digestion is complete 100 c.c. of iodine-free water are cautiously added. This is slowly poured directly onto the digest. Thorough mixing is thus assured. As soon as bubbling has ceased the funnel is inserted and the condenser is connected by means of the connecting arm. The receiving flask containing 1 c.c. of molar potassium hydroxide is placed so that the condenser tip is immersed in alkali. Six cubic centimeters of 50 per cent phosphorous acid are placed in the funnel and cautiously run in, the funnel then being rinsed with 30 c.c. of iodine-free water which is cautiously run into the digest. The

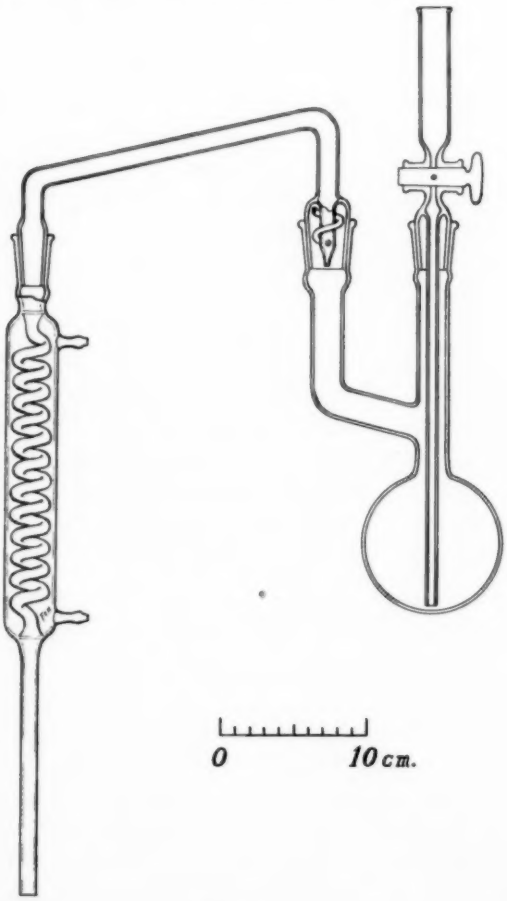

Fig. 1.

Fig. 2.

stopcock is closed and heating begun, gently for two or three minutes until boiling is started, then rapidly. It is important not to allow the digested mixture to cool before distillation is begun. Distillation is continued until white fumes can be seen in the top of the condenser, at which time about 100 c.c. of distillate have come over, this taking about thirty minutes. The volume of distillate has meanwhile been kept at about 5 c.c. by boiling it off as it is received. The receiving flask is now lowered, heating is discontinued, the connecting arm is removed from the condenser which is then rinsed inside and at the tip with a few cubic centimeters of iodine-free water.

The volume of distillate is made up to 15 or 20 c.c. by rinsing down the walls of the receiver. One drop of 0.0007 molar methyl orange is added, and then 3 per cent sulphuric acid, drop by drop, until the color is a clear pink, not orange pink. Bromine vapor is poured from a bromine-containing bottle into the solution which with a little stirring assumes a light yellow color. This bromination is carried out in another room. Excess bromine is boiled off by heating in a fume hood and the solution is boiled down to about 2 or 3 c.c. It is then placed in a refrigerator for five minutes where it cools to about 10° C. Four drops of 1 per cent potassium iodide and 1 drop of 1 per cent starch are added. After wetting the walls of the flask with the solution, it is titrated with 0.0005 N sodium thiosulphate delivered in 0.005 c.c. portions from a 0.2 c.c. serum pipette graduated in thousandths of a cubic centimeter and immersed in the solution being titrated.

The procedure requires about two hours for analysis of 10 c.c. of blood.

Urine is analyzed in samples containing a convenient amount of iodine for titration, usually 25, 50, or 100 c.c. With the smallest samples no reduction in volume is necessary, but to the larger samples measured into the digestion flask 1 c.c. of saturated potassium hydroxide is added and the mixture carefully boiled down to about 10 c.c. The inside of the neck of the flask is rinsed down with 10 c.c. of iodine-free water. The remainder of the procedure is as for blood, the quantities of chromic and sulphuric acids used depending on the character of the urine. Ten cubic centimeters of chromic acid solution have been sufficient for 100 c.c. samples of human urine.

Feces are dried and analyzed in 1 gm. or 0.5 gm. samples. The former have been treated with 12 c.c. of chromic acid solution.

TABLE I

DETERMINATION OF IODINE IN SEVERAL MATERIALS

SUBSTANCE ANALYZED	PRESENT (GAMMA)	IODINE FOUND (GAMMA)	ERROR (GAMMA)	PER CENT RECOVERED
KI	0.81	0.74	−0.07	91
KI	0.81	0.64	−0.17	79
KI	0.81	0.79	−0.02	97
KI	0.81	0.74	−0.07	91
KIO_2	0.48	0.42	−0.06	88
KIO_3	0.48	0.42	−0.06	88
KIO_3	0.48	0.45	−0.03	94
KIO_3	0.48	0.29	−0.19	60
10 c.c. beef blood		0.50		
10 c.c. beef blood		0.56		
10 c.c. beef blood		0.50		
10 c.c. beef blood		0.45		
10 c.c. beef blood		0.50		
10 c.c. beef blood		0.61		
10 c.c. beef blood		0.50		
10 c.c. beef blood		0.61		
Average		0.54		
10 c.c. beef blood + 0.81 gamma KI	1.35	1.40	+0.05	104
10 c.c. beef blood + 0.81 gamma KI	1.35	1.30	−0.05	96
10 c.c. beef blood + 4.05 gamma KI	4.59	4.58	−0.01	100
10 c.c. beef blood + 4.05 gamma KI	4.59	4.68	+0.09	102

Results are calculated by this formula:

(x) c.c. 0.0005 N thiosulphate = 10.6 (x) gamma iodine in material analyzed.

A volume correction (Harvey, 1935; Fashena and Trevorrow, 1936) for the final titration which is carried out in north light transmitted through white paper has not been employed. A series of experiments on this titration performed under the conditions used in this procedure have shown that up to a volume of 3 c.c., the corrections suggested are not properly applicable here.

The use of greater quantities of potassium iodide than those found to be sufficient by Allott, Dauphinee and Hurtley (1932) and Korenman (1934) may, as the former demonstrated, lead to erroneously high values. This is particularly true if the acid solution containing the hydriodic acid is much exposed to atmospheric oxygen before titration.

TABLE II

CONDITION OF PERSONS	GAMMA OF IODINE PER 100 C.C. OF BLOOD
Normal	6.9, 6.4, 5.8, 5.8, 5.6, 5.4, 5.3, 4.3, 4.2, 4.0, 4.0, 4.0, 3.9, 3.6, 3.3 (average, 4.8)
Nodular colloid goiter	11.1, 9.3, 9.3, 8.0, 7.3, 6.4, 6.3, 6.2, 5.9, 5.4, 5.3, 5.2, 3.9, 3.6, 3.4, 3.3
Toxic nodular colloid goiter	11.4, 8.7, 8.5, 6.2, 5.8, 4.3
Adenoma of thyroid	23.0, 5.7, 5.3, 3.2
Benign fetal adenoma of thyroid	14.3, 11.1, 6.6, 6.0, 5.0, 4.6
Toxic adenoma of thyroid	13.1, 12.9, 4.8, 4.4, 4.4
Hyperplasia of thyroid	16.4, 11.4, 9.3, 8.6, 7.7, 4.6, 3.9, 3.6, 3.3
Same with exophthalmos	27.1, 4.6, 4.0
Hyperthyroidism	11.9, 6.4, 3.2
Hypothyroidism	2.1
Chronic cholecystitis with stones	2920, 27.5, 5.5, 4.9, 4.7, 4.6, 4.5, 4.2, 2.5
Same, over three weeks postoperative	18.7 (preoperative, 500), 13.0
Chronic cholecystitis, subacute	5.9, 3.9, 2.9
Chronic cholecystitis with cholesterosis of gallbladder	110
Same, after glucose administration	87
Chronic cholecystitis with neurosis	4.7
Chronic cholecystitis with appendicitis	180
Carcinoma of gallbladder	19.6
Carcinoma of liver	5.3
Portal cirrhosis of liver with ascites	8, 6.0
Acute hydrops of gallbladder	4.2
Acute cholangeitis	49.5
Carcinoma of stomach	4.0
Tumor of pancreas	6.1
Tumor of breast	4.8, 3.6
Carcinoma of lymph gland	4.3
Endocervicitis, endometritis, salpingitis and appendicitis	2.9
Fibroid uterus	4.6, 3.3
Cancer of uterus	5.7
Uterine polyp	6.9, 6.2, 3.6
Ovarian cyst	5.9, 5.0, 4.6, 3.3
Inguinal adenitis	5.3
Epigastric hernia	5.0
Umbilical hernia with ascites	6.0
Prolapsed rectum with hemorrhoids	4.3
Gastric hyperacidity	4.6
Intercostal neuritis	3.7
Facial neuralgia	5.6
Epilepsy	6.6
Acute bronchitis	6.9
Lymphatic leucemia	5.2
Chronic appendicitis	5.0

A Summary of Clinical Blood Iodine Findings

Twenty-two cubic centimeters of blood drawn with proper precautions to avoid iodine contamination were placed in a bottle containing 50 mg. of dry, purified potassium oxalate. This was then kept in a refrigerator until analyzed by the method already described.

From the following summary of values persons known to have had iodine medication within the nine days* preceding the drawing of the sample, female patients during or within a few days of menstruation or pregnant, and patients with complicated or uncertain diagnoses have been omitted. All the figures in Table II but 34 are averages of two or more analyses, usually carried out on 10 c.c. samples of blood.

SUMMARY

A simplified method for the determination of iodine in biological materials is described. With the simple apparatus used, the iodine in 10 c.c. of blood may be determined in two hours with no greater error than attends the best micromethods hitherto published.

REFERENCES

Allott, E. N., Dauphinee, J. A., and Hurtley, W. H.: The Determination of Small Quantities of Iodine in Blood, Biochem. J **26**: 1665, 1932.

Fashena, G. J., and Trevorrow, V.: A Note on the Determination of Iodine in Biological Material, J. Biol. Chem. **114**: 351, 1936.

Harvey, C. O.: The Determination of Iodine in Biological Substances, Med. Research Council, Special Rep. Series, No. 201, 1935.

Korenman, I. M.: Über die Empfindlichkeit der Iodstärkereaktion, Mikrochemie **15**: 25, 1934.

Leipert, T.: Die Bestimmung kleinster Iodmengen in organischem Material, Biochem. Ztschr. **261**: 436, 1933.

Idem: Zur Kenntnis des physiologischen Blutiodspiegels, Biochem. Ztschr. **270**: 448, 1934.

Trevorrow, V., and Fashena, G. J.: The Determination of Iodine in Biological Materials, J. Biol. Chem. **110**: 29, 1935.

*This is probably insufficient time to allow in some gallbladder cases.

A SIMPLE, PRACTICAL AND STERILE TECHNIQUE FOR OBTAINING BONE MARROW FROM ANIMALS BY PUNCTURE*

LOWELL A. ERF, M.D., CINCINNATI, OHIO

SINCE the work of Arinkin[1] (1927), who advocated sternal puncture for repeated examination of human bone marrow, there has been increasing evidence that marrow obtained by puncture has many advantages and few disadvantages.[2-4] The disadvantages of losing the marrow architecture is overshadowed by such advantages as (a) the possibility of frequent qualitative examinations of the bone marrow, and (b) the vastly more distinct differentiation of the marrow cells. (Fresh bone marrow cells spread by smear or filmed by supravital technique can be differentiated much better than cells prepared in marrow tissue sections.)

The following technique may be used on small experimental animals (rat, rabbit, dog, etc.) and requires the use of only three instruments, a syringe, a

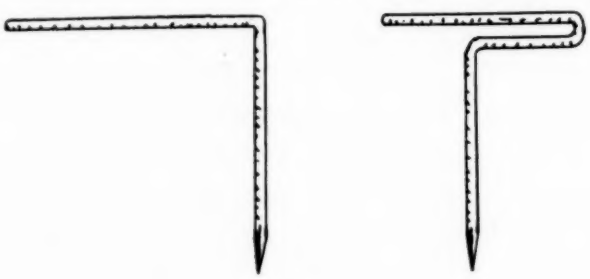

Fig. 1.

lumbar tap needle, and a Steinman pin. The spinal puncture (Quincke type) needle is preferably shortened to 1½ inches. The Steinman pin, which is the usual steel wire pin used in orthopedic surgery, is about ¼ inch in diameter and six inches long, tapered to a sharp point. This pin is bent at right angles at the middle of the shaft in order that the pin may be grasped and rotated by hand (Fig. 1).

Technique.—The hair is shaved, or is well moistened with alcohol, over the inner aspect of a tibia, femur or humerus of the anesthetized animal. The Steinman pin is then inserted directly through the skin (without an incision of the skin), through the muscles and fascia, through the periosteum, to the *midline* of the shaft of the bone at a site where the selected bone is near the surface and, where there are no large blood vessels nor nerves to be punctured or injured. The pin is then rotated back and forth after the point of the pin has been securely pushed into the bony cortex so that on initial rotation it does not slip off and injure the surrounding tissues. After the pin has been bored

*From the Department of Hematology, Institute for Medical Research, Jewish Hospital.

(at right angles) part way through the cortex, it is slanted to nearly a 30° angle and bored through to the medullary cavity. (This allows the needle which is inserted later to proceed a short distance within the medullary cavity.) One can "feel" when the pin has reached the medullary cavity after a little experience, and will know definitely when the other side of the shaft or endosteum is struck. The pin should be carefully and slowly removed so that the perforated skin and tissues will remain directly over the bony perforation. (If the skin does move, the bony perforation usually can be palpated.) There is little or no bleeding since the traumatized tissue juices rapidly coagulate the escaping blood. A lumbar puncture needle (with stylet) is then inserted to the marrow cavity. The stylet is withdrawn and a syringe attached. As suction is applied, the needle is thrust forward and rotated so that a plug of marrow will fill the bore of the needle.[5] Most animal marrows have a consistency somewhat similar to liver and cannot be aspirated as a fluid. Occasionally some bloody fluid may reach the syringe but this is not desired. Release the vacuum of the syringe and detach the syringe from the needle (leaving needle in the marrow cavity). The needle is then removed. The needle bore contains the plug of marrow which is expelled by replacing the stylet. Nothing is done to the puncture wound. There are no complications, postpuncture, unless a large vessel or nerve has been injured. This is avoided by knowing the anatomy of the usual puncture sites.

The marrow plug obtained can be spread on slides, filmed by the supravital technique or can be fixed and sectioned. The qualitative changes can be estimated, as in the illustrated case below. A rabbit with marrow punctures of the humerus, femur, and tibia was biopsied, within an hour following the punctures, just proximal to the puncture sites. A comparison of the differentials is shown in Table I.

TABLE I

	HUMERUS		FEMUR		TIBIA	
	BIOPSY	PUNCTURE	BIOPSY	PUNCTURE	BIOPSY	PUNCTURE
Erythroblasts	5	7	4	3	3	1
Normoblasts	54	80	37	73	50	45
Hematogones		4	2			3
Myeloblasts	8	9	10	7	7	11
Myelocytes neut.	4	14	6	11	18	12
Nonseg. neut.	24	22	17	27	10	22
Segmented neut.	45	35	51	36	44	41
Myelocytes eos.				1		
Eosinophiles		1				
Myelocytes baso.		1			1	
Basophiles	2		1	2	4	
Monocytes			2		1	1
Reticulum cells	2	2	1		1	
Lymphocytes	14	9	9	14	12	10
Plasma cells	1	2	1	2	1	
Megakaryocytes		1			1	

SUMMARY

A rapid simple technique which permits repeated qualitative studies of the marrow of small animals is described.

REFERENCES

1. Arinkin, M.: Methodology of Examining Bone Marrow in Live Patients With Hematopoietic Diseases, Vestnik Khir. **10**: 57, 1927.
2. Young, R. H., and Osgood, E. E.: Sternal Marrow Aspirated During Life; Cytology in Health and Disease, Arch. Int. Med. **55**: 186, 1935.
3. Segerdahl, E.: Ueber Sternalpunktionem, Acta. Med. Scandinav. Supp. **64**: 1, 1935.
4. Nordenson, N. G.: Sternal Puncture, Stockholm, Bortzells, Esselte, 1935.
5. Martin, H., and Ellis, E.: Needle (Aspiration) Biopsy, Surg. Gynec. Obst. **49**: 578, 1934.

AN OXYGEN ANALYZER FOR USE IN OXYGEN TENT THERAPY*

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THE apparatus described is an inexpensive, easily constructed oxygen analyzer intended for use with the oxygen tent. Its operation is so simple that the average ward attendant can make a quantitative determination of the oxygen concentration within the oxygen tent in about one minute. A single demonstration is all the instruction needed. The apparatus is designed for use on the hospital ward. It is not recommended for laboratory use.

In principle the method consists of absorbing the oxygen from a known quantity of gas and determining the residual volume. The oxygen is absorbed by metallic copper in the presence of ammonium hydroxide and ammonium chloride, the end-products being a complex cupric ammonium chloride and water.

Extreme accuracy is sacrificed in favor of simplicity. Analysis of 30 specimens of gas varying in oxygen content from 100 per cent to 20 per cent showed an error range extending from minus 3 per cent to plus 2 per cent. The average error was minus 0.9 per cent. The apparatus contains sufficient reagent to permit approximately 300 average tests without replacement of solution. In a continuous test run, 1,300 c.c. of pure oxygen were absorbed by the apparatus before absorption was retarded. In spite of the retarded absorption after 1,300 c.c., accurate results were still obtained up to 1,600 c.c.

The structure of the apparatus and the details of its construction can best be understood by referring to the diagrams and accompanying notes.

(*A*) Mohr's pinchcock stapled to wood block (see Fig. 2). Place staples high on pinchcock ring so that spring is not interfered with. Attach block to mounting board close enough to make entry tube curve forward, but not close enough to kink tube. (*B*) Entry tube, a three-inch length of blood pipette tubing. (*C*) DeKhotinsky cement seal. (*D*) Barrel of 30 c.c. syringe. (The absorption chamber.) The 20 c.c. size will do also. (*E*) Snugly rolled common copper screening. Do not roll too tightly or sediment will clog absorption chamber prematurely. Flame screening before use to remove lacquer. Push roll up to shoulder of syringe. (*F*) Rubber stopper. Cut stopper in half and push

*From the Veterans' Administration Facility,

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Received for publication, November 30, 1936.

in till outer surface is $\frac{3}{16}$ inch within rim of (*D*). This permits firmer seal. (*G*) DeKhotinsky cement seal. (*H*) Eye dropper tube with flange upward.

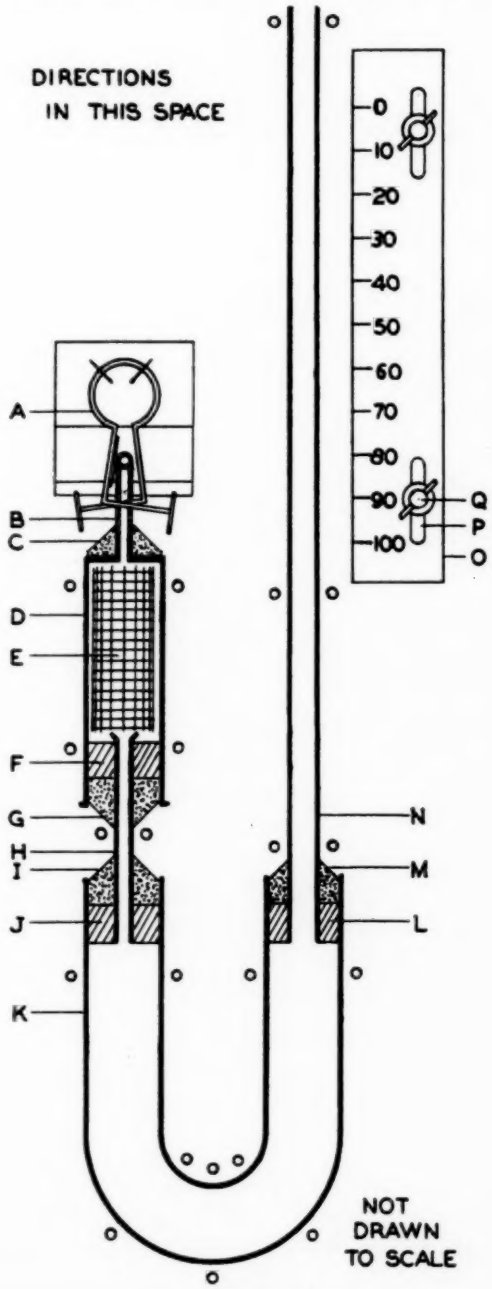

Fig. 1.—The analyzer. .

When reconditioning is necessary this makes removal of stopper (*F*) easy. (*I*) DeKhotinsky cement seal. (*J*) Rubber stopper. Cut in half and push below rim of (*K*) for strong seal. Note that filed end of eye dropper tube is flush

with bottom of stopper to prevent air trap. (*K*) Calcium chloride "U" tube (150 mm. size). This is the reagent reservoir. (*L*) Cut rubber stopper. Same as (*J*). (*M*) DeKhotinsky cement seal. (*N*) Glass tubing (manometer) 10 mm. outside diameter, 18 inches long. Note end is flush with stopper to prevent gas trap. If diameter of tube is smaller it should be proportionately longer. The tube is purposely made excessively long because the operator will occasionally attempt a test without having first released the residual gas from a previous test. The excess length prevents spilling of the reagent in this event. (*O*) Discussed under Calibration. (*P*) Discussed under Calibration. (*Q*) Discussed under Calibration.

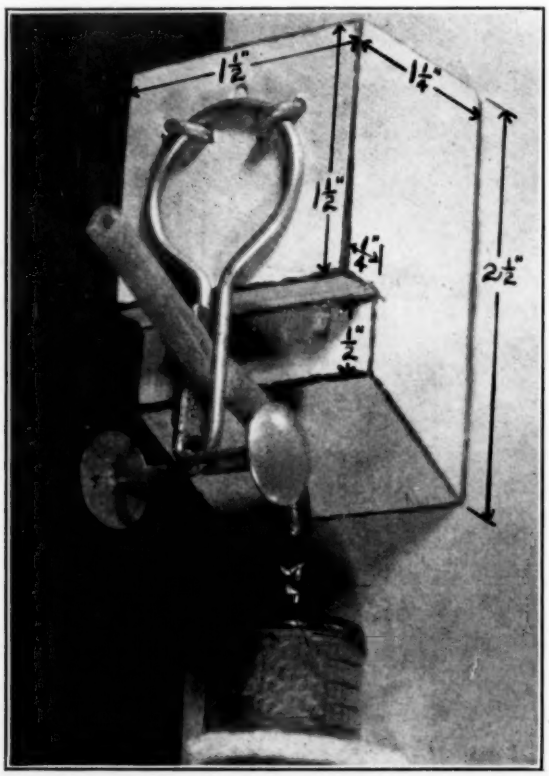

Fig. 2.—Perspective of entry tube pinchcock assembly with dimensions of wood block. Note bevel of lower end of block.

Assemble as much of the apparatus as is convenient and heat to about 100° C. in an oven. Then complete assembly and make the three DeKhotinsky cement seals. These seals are best worked with a small electric soldering iron. If unfamiliar with DeKhotinsky cement, it will be wise to read directions accompanying cement carefully and experiment with glass to rubber seals before attempting to work seals on the apparatus. Medium or hard grades of DeKhotinsky cement are satisfactory. An effort should be made to keep the elements of the apparatus in good alignment, but slight deviations will not affect the efficiency of the apparatus.

The assembly is mounted on a suitable board with the manometer tube either on the right or left depending upon the position which gives the snuggest fit. Punch holes are drilled in the mounting board at the points indicated by small circles on Fig. 1. Use the assembly as a guide in laying out these holes. The assembly is bound to the mounting board with common pipe cleaners through the holes and twisted on the back. If desired, the apparatus may be mounted in a box with hinged or removable lid for protection.

CALIBRATION

To calibrate the instrument, wedge pinchcock open and fill with water by way of the long tube until the fluid level is about ¼ inch above the level of the shoulder of the absorption chamber. Shake vigorously for a minute and let apparatus stand for about ten minutes to insure complete escape of air. Then close pinchcock and mark fluid level with a sharp crayon, or gummed paper. Take 10 c.c. of air into a syringe, attach syringe tip to entry tube, open pinchcock and expel entire sample into the apparatus. Close pinchcock before removing syringe. Mark new fluid level. Open pinchcock to permit escape of air. Check these marks with at least ten additional 10 c.c. samples of air using several different syringes. Discard the water. The lower mark represents the 100 per cent level, the upper mark the 0 per cent level. Measure distance between marks. Lay off this distance on a small strip of wood (*O*) which is to serve as the slide rule. Subdivide into ten equal spaces, each of which represents 10 per cent. Further subdivisions may be made if desired. Two slots (*P*) are cut into rule to permit adjustment of scale to fluid level. Wing nuts (*Q*) hold scale in place.

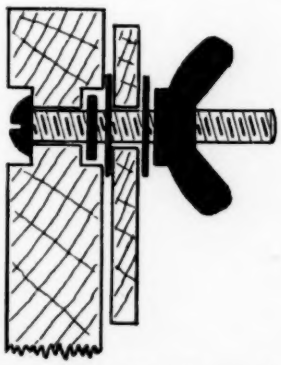

Fig. 3.—Detail of cross-section through slide rule assembly. Wood parts from left to right are: *1*, Mounting board drilled for bolt and countersunk back and front for bolt head and locking nut; *2*, slotted slide rule. Metal parts from left to right are: *1*, Bolt; *2*, locking nut; *3*, washer; *4*, washer; *5*, wing nut.

ABSORBING FLUID

A modified Badger's reagent is used as absorbing solution. This consists of one part stronger ammonia water (28 per cent) in two parts saturated ammonium chloride solution.

The apparatus as described will hold from 90 c.c. to 110 c.c. of this reagent. Fill apparatus with reagent until the fluid level is about ¼ inch above level

of shoulder of the absorption chamber. Make certain that all the air is expelled by shaking and letting stand for a short while with the pinchcock open. Then close pinchcock. Adjust slide rule so that the 100 mark is at the fluid level. The apparatus is now ready for use.

The following optional absorbing fluids may be used. The original Badger's reagent, recommended for oxygen concentrations of 90 per cent or more, is made by saturating equal parts of concentrated ammonium hydroxide and water with ammonium chloride. The fluid used by Riseman and Lesnick, recommended

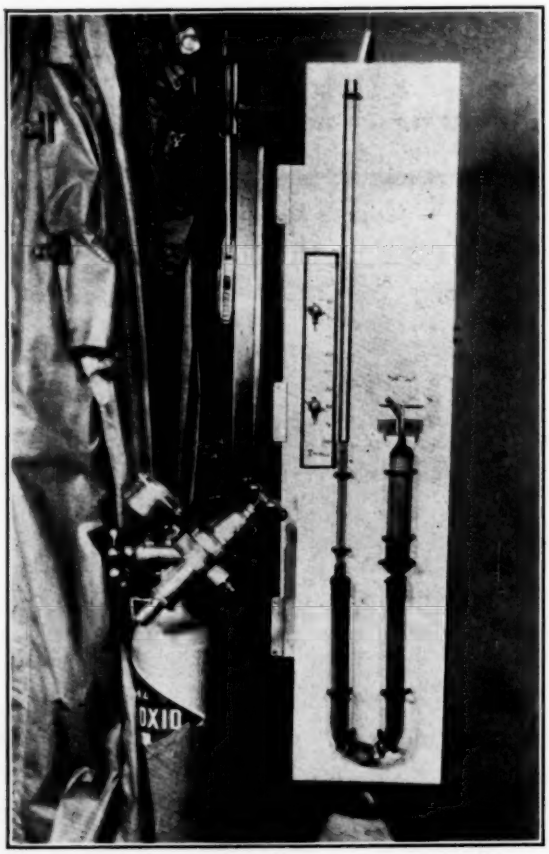

Fig. 4.—The analyzer attached to oxygen tent.

for low oxygen concentrations, is made by dissolving 130 gm. of ammonium nitrate in 90 c.c. of water to which is then added 30 c.c. of concentrated ammonium hydroxide.

DIRECTIONS

The following instructions as to the operation of the apparatus should be typed on a card, protected by shellac, and tacked to the mounting board.

1. See that solution in scale tube is slightly above level of shoulder of the absorption chamber. If not, add absorbing fluid with dropper.
2. Set sliding scale with 100 at fluid level.

3. Collect 10 c.c. specimen of gas in oxygen tent with 10 c.c. syringe.

4. Attach syringe to entry tube, open pinchcock, expel specimen into apparatus, close pinchcock, and remove syringe.

5. Read oxygen percentage when fluid level stops falling.

6. After reading, open pinchcock to release residual gases. Apparatus is now ready for next test.

CLEANING APPARATUS

When delayed absorption is noted the apparatus should be cleaned as soon as convenient. The entry tube is pulled out of pinchcock, the expended fluid is drained and discarded and the apparatus is well flushed with running water by way of the entry tube. The flush water is then drained off. Invert the apparatus so that the entry tube is down. With a 20 c.c. syringe, douche out the absorption chamber with two or three changes of half strength ammonia water (14 per cent) until the blue color becomes faint or disappears. Flush out again with water, drain, and refill with fresh reagent. The apparatus is now ready for use again.

The copper screening lasts indefinitely. If, however, it is necessary to renew the screening, only the seal at (*G*) need be broken.

REFERENCES

1. Badger, W. L.: Determination of Oxygen by the Copper-Ammonia-Amonium-Chloride Reagent, Indust. & Engin. Chem. **12**: 161, 1920.
2. Riseman, J. E. F., and Lesnick, G.: A Simple Method of Oxygen Analysis for Use in Oxygen Tent Therapy, New England J. Med. **215**: 65, 1936.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

DIPHTHERIA, Types of C. Diphtheriae in Maryland, Perry, C. A., Whitley, O. R., and Petran, E. Am. J. Hyg. 23: 580, 1936.

A study has been made of the so-called gravis, mitis, and intermediate types of *C. diphtheriae* in Maryland.

All cultures of *C. diphtheriae* were found to produce acid from dextrose and dextrin, but not from sucrose. Of 50 strains classified as gravis, 33 (66 per cent) produced acid from glycogen and starch, 8 (16 per cent) produced acid from starch but not glycogen, while 9 (18 per cent) produced acid from neither starch nor glycogen. None of the strains classified as mitis or intermediate produced acid from glycogen or starch except 3 mitis strains which gave feeble acid reactions in starch.

The type of growth in both was not found constant for any of the three types. Growth of gravis strains in both appeared usually as a granular to negative turbidity with a heavy to moderate granular pellicle, that of the mitis type usually as a uniform or granular turbidity with a light or moderately heavy pellicle, and that of the intermediate as a granular or negative turbidity with a light pellicle.

A high degree of correlation has been found between cellular morphology and the three types. This was much more distinctive when cultures were grown on Douglas' tellurite medium than when they were grown on Loeffler's medium. Cellular morphology has been found an easy and dependable way for an experienced worker to routinely classify strains of *C. diphtheriae.*

Virulence was determined by intradermal inoculation of saline suspension into guinea pigs. Only slightly more than 3 per cent of strains were found avirulent when repeated tests were made. No intermediate strains were found avirulent while 2 out of 50 gravis strains and 8 out of 132 mitis strains were. Twenty-four per cent of the strains were from carriers.

During 1934 and 1935, all three types of *C. diphtheriae* were found in 10 of the 23 counties of Maryland. The gravis type was found in 11 counties, the mitis in 21, and the intermediate in 13. Epidemics involving considerable numbers of cases and carriers of both the mitis and intermediate types occurred, but only small epidemics and scattered cases of the gravis type were observed.

No evidence of natural dissociation was observed. Repeated isolations were made from both cases and carriers until released as negative, yet no change of type was ever noted. For practical purposes the types may be considered stable.

No great difference in the severity of clinical diphtheria caused by the three types has been found.

DIABETES MELLITUS, Daily Variation of Sugar Content of Blood and Urine During Treatment of, Rogers, F. L. Arch. Int. Med. 57: 1027, 1936.

A study of 24 cases of diabetes mellitus with regard to a daily quantitative variation in sugar content of the blood and in the amount of sugar excreted showed such a variation in 16 cases. In 9 cases the variation was slight; in 7 it was marked enough to interfere with satisfactory treatment when the injections of insulin were given before meals. The variation consisted in an early morning rise in the blood sugar content, a parallel rise in the amount of sugar excreted and an afternoon fall in both. Meals did not seem to affect the curves. As a rule the variation was found in young patients with severe diabetes, while older patients with milder forms of the disease often failed to show it.

The administration of insulin in anticipation of the early morning rise has resulted in more satisfactory control than could be obtained otherwise, but there was no evidence that

the total requirement of insulin could be reduced by this method of administration, as stressed by Mollerstrom. A single dose of insulin given in anticipation of the peak failed to control the condition adequately throughout the twenty-four hours.

One patient showing the variation was restudied after a year, and similar variability was found, indicating permanence.

A single estimation of blood sugar, regardless of the time of day, gives incomplete information and may be misleading.

Accurate establishment of the existence of a definite and constant early morning rise in the blood sugar level always should precede the giving of large doses of insulin in the early morning.

PREGNANCY, Test for: Demonstrating the Presence of Histidine in the Urine, Renton, H. South African Med. J. **9**: 441, 1935.

Renton describes a new test for pregnancy devised by Kapeller-Adler and based upon the presence of histidine in the urine. Renton believes the test to be quite reliable.

Reagents:

1. Bromine reagent:

Pure bromine	1 c.c.
Glacial acetic acid	100 c.c.
Distilled water	300 c.c.

2. Alkali reagent:

Ammonium carbonate	10 gm.
Distilled water	90 c.c.
Pure liquid ammonia	200 c.c.

3. Potassium iodide-starch paper.

The bromine reagent is quite stable, but should be carefully sealed for storage.

Method.—Five cubic centimeters of filtered urine are placed in a test tube, and bromine reagent is added by means of a pipette until the mixture becomes light yellow in color. Usually about 2 or 5 c.c. of the reagent are necessary, sometimes more. This is the most intricate part of the test, as it is necessary to have a minute excess of bromine in the urine before proceeding with the next step in the test. The urine "consumes" the bromine, and the excess of bromine is tested for with the starch paper. The best way to do this is to cut a series of small squares of the white paper and place them on a piece of glass or on the bottom of a white dish. By means of a glass rod, a drop of the mixture is placed on a piece of the paper. Free bromine gives a violet color. If there is too much bromine, the color will be dark violet. The object is to obtain a pale violet reaction, indicating minute excess of bromine. This part of the test is most important, and if not carried out with care, will prove a source of error.

If the color is found to be too dark, a few more drops of urine are added, until the correct color is obtained. Similarly, if the color is too pale, more bromine reagent is added drop by drop, in the same manner. It is necessary that this color reaction should remain stable for ten minutes. Experiment will show that even if the color reaction is deep violet at first, it may be quite absent in a few minutes. If there is not sufficient free bromine, this will be "consumed" by the urine and the color reaction will not remain. This adjustment of the color requires a little practice, but the ability to adjust the quantities is soon acquired.

When this color reaction has been settled, i.e., after a lapse of ten minutes, 3 c.c. of the alkali reagent are next added and the mixture shaken. The mixture is then placed in a steaming water-bath for three minutes, and if the test is positive for histidine and pregnancy, a mauve color deepening to reddish purple, depending on the amount of histidine present, will result. This usually becomes more marked on standing for a few minutes.

Best results are obtained by using a twenty-four hour specimen of urine, but if this is not possible, then a morning specimen of good concentration should be used. A very dilute urine will give inconclusive results.

CHANCROIDS, Intradermal Reaction for, With Chancroidal Bubo Pus, Cole, H. N., and Levin, E. A. J. A. M. A. **105**: 2040, 1935.

The use of an intradermal diagnostic test, with sterilized pus from chancroidal buboes, confirms the specificity of the test in cases of Ducrey bacillus infection and corroborates the observations of earlier workers.

The interval from the presence of the ulceration to the production of a positive test was on the average five weeks.

The presence of a chancroidal bubo seems to increase the rapidity and the intensity of the intracutaneous test.

The allergic properties raised in a patient by a chancroidal infection persist for years, in our experience at least from thirty to thirty-four years.

ALCOHOLISM, Acute, Treatment of, Robinson, L. J., and Selesnick, S. J. A. M. A. **105**: 1734, 1935.

Acute alcoholic coma with dangerous respiratory depression, paralysis, and cyanosis is a medical emergency. Death may be definitely prevented and recovery accelerated by inhalation of a mixture of 10 per cent carbon dioxide and 90 per cent oxygen for a length of time sufficient to reestablish and maintain normal respiration and color even after the inhalation is suspended.

A minimum time of half an hour should be observed. If necessary, the inhalation may be carried out longer.

An accelerated decrease in venous blood alcohol levels is produced by carbon dioxide-oxygen inhalation over a period of thirty or more minutes, indicating an accelerated decrease in total body alcohol.

No significant blood sugar observations were recorded either before or after carbon dixide-oxygen therapy.

In alcoholic intoxication there was a tendency to lowered blood carbon dioxide capacity. No appreciable change and no carbon dioxide retention were produced by carbon dioxide-oxygen therapy.

The blood lactic acid content was elevated in alcoholism but was unaffected by carbon dioxide-oxygen therapy.

The purpose of the carbon dioxide-oxygen therapy is not to arouse completely a comatose alcoholic patient but to reduce him from a state of dangerous paralytic alcoholism to the less deep stage of anesthesia from which he can safely be expected to recover.

The therapy is recommended as an emergency treatment and is not indicated in the general run of moderately intoxicated patients so frequently encountered.

BLOOD SEDIMENTATION: Effect of Anticoagulant on, Greisheimer, E. M., Hodapp, A., and Goldsworthy, E. Am. J. M. Sc. **190**: 775, 1935.

Although the changes in the sedimentation rate are probably not great enough to affect the clinical results markedly, the following observations are interesting. Decrease in the amount of solvent hastens sedimentation; one can expect a faster rate when the anticoagulant is used dry. An increase in the amount of solvent beyond the control amount retards sedimentation, although the blood is diluted. An increase in the amount of anticoagulant, without change in the amount of solvent, tends to retard sedimentation. Ammonium citrate increases the rate in the Cutler and decreases it in the Westergren. Sodium oxalate, potassium oxalate, and ammonium oxalate tend to increase the rate in the Cutler method, in the above order. The first decreased the rate in the Westergren, the second had no effect and the third increased the rate. Heparin increases the rate in all cases, unless used with a greater amount of the solvent than the control.

Sodium citrate is a highly satisfactory anticoagulant for use in the sedimentation test.

Fifteen milligrams in 0.5 c.c. of distilled water, or 0.5 c.c. of 3 per cent sodium citrate, is sufficient to prevent coagulation in 4.5 c.c. of blood; this amount does not hemolyze the red blood corpuscles. A change either in the amount of anticoagulant or in the amount of solvent alters the rate of sedimentation.

MONONUCLEOSIS, Infectious: The Nature of the Heterophilic Antibodies in, Davidsohn, I., and Walker, P. H. Am. J. Clin. Path. **5**: 455, 1935.

The antibodies (agglutinins and hemolysins for sheep cells) in infectious mononucleosis are heterophilic in nature, but are not of the Forssman heterophilic type.

The erythrocytes of the sheep and the kidney of the horse contain at least two common heterophilic antigens: (a) The Forssmann heterophilic antigen; (b) the heterophilic antigen that reacts with the heterophilic antibodies found in infectious mononucleosis.

The absorption of a specimen of serum with the kidney of the guinea pig and of another sample of the same serum with the kidney of a rabbit, offers a convenient method for the differentiation of infectious mononucleosis from serum disease, and for the diagnosis of borderline cases. The agglutinins for sheep cells will be almost completely absorbed by the kidney of the guinea pig but only partly by the kidney of the rabbit in cases of serum disease and in cases that are not infectious mononucleosis, while in infectious mononucleosis the absorption with both tissues will be only partial and about equal in degree.

TUMORS, Radiosensitivity of, Desjardins, A. U. J. A. M. A. **105**: 2153, 1935.

Classification of Tumors from Standpoint of Radiosensitiveness.

RADIOSENSITIVE TUMORS

Group I
- Lymphoblastoma
  - Lymphosarcoma
  - Hodgkin's disease
- Lymphoepithelioma
- Diffuse epithelioma (Ewing), or endothelial myeloma (Kolodny) of bone

Group II
- Embryonal carcinoma (seminoma of testis) (Spermatogonial cells)
- Embryonal carcinoma of ovary (follicular epithelium)
- Embryonal carcinoma of kidney (Wilms tumor)

Group III
- Giant cell tumor of bone
- Multiple myeloma of bone
- Basal cell epithelioma
- Transitional cell epithelioma
- Mucinous carcinoma of intestinal tract (mucus-secreting epithelial cells)
- Hemangioma
- Hemangioendothelioma

MODERATELY RADIOSENSITIVE TUMORS

Group I
- Carcinoma of uterine cervix
- Carcinoma (not adenocarcinoma) of thyroid gland

Group II
- Epithelioma of tonsil and pharynx
- Carcinoma of breast
- Epithelioma of bronchus
- Epithelioma of lip and eyelids
- Epithelioma of mouth and tongue
- Epithelioma (squamous cell) of skin
- Carcinoma of rectum
- Chondrosarcoma of bone

RADIORESISTANT TUMORS

- Fibromyoma (uterus) direct irradiation
- Fibrosarcoma
- Carcinoma of esophagus and stomach
- Adenocarcinoma anywhere, with possible exception of uterus (cervix or fundus) and thyroid gland
- Mixed tumor of parotid gland
- Hypernephroma
- Sarcoma of bone, osteogenic
- Melanoblastoma and nonmelanotic melanoblastoma of skin
- True teratoma
- Neurofibroma
- Fibroma
- Chondroma
- Lipoma
- Myxoma
- Myxosarcoma

CHOLESTEROLURIA, in Bright's Disease, Bruger, M. Am. J. Clin. Path. 5: 504, 1935.

Cholesterol is excreted normally in the urine in amounts averaging 0.5 mg. per day. In patients with Bright's disease, the maximal excretion observed was 92 mg. per day.

In the urine of patients with Bright's disease, the cholesterol excretion was found to parallel the protein excretion.

Hypercholesterolemia, per se, does not induce cholesteroluria; however, when proteinuria exists, more cholesterol is excreted per gram of protein in the presence of a higher blood cholesterol than when the blood cholesterol is at a lower level.

The urinary sediment may contain as much as 40 per cent of the total cholesterol in the urine.

The most significant finding in this investigation has been the observation that the cholesterol excretion in the urine has been found to parallel the protein excretion. In patients with nephrosis and nephrotic glomerular nephritis, a marked loss of protein in the urine is accompanied by an excessive excretion of cholesterol. The highest output observed was a loss of 92 mg. of cholesterol in twenty-four hours. At this rate of excretion, and providing the cholesterol in the blood is not replenished, the total blood cholesterol can be depleted within a few months. Obviously, there are processes which compensate for such a marked loss of cholesterol from the organism.

In view of these observations, it is reasonable to assume that the blood cholesterol is maintained when hypercholesteroluria exists by one or more of such compensatory processes as: increased cholesterol synthesis, decreased cholesterol destruction or diminished excretion through the intestines. A similar thesis to account for the conservation of cholesterol in the organism in other pathologic conditions associated with marked losses of cholesterol has been proposed.

BRUCELLOSIS, Treatment of Acute and Chronic, Angle, F. E. J. A. M. A. 105: 939, 1935.

The author comments upon the fact that up to Jan. 1, 1935, 9,965 cases of this disease have been reported in the United States. The disease is evidently increasing and, without doubt, there are many unrecognized and untreated cases. For this reason Angle recommends that an agglutination test be done routinely on all Wassermann serums and suggests, also, that a macroscopic agglutination test (slide, drop method) might well be included in the physician's study of patients. He also believes that if a proper study is made Brucella may be found as the etiologic organism in many so-called sterile abscesses. As agglutinins may be absent in the blood in 5 per cent of cases, Angle uses an intradermal test for the recognition of such cases, using any commercial Brucella vaccine as the antigen and injecting 0.02-0.04 c.c. intradermally. It is advisable to use as a control another patient or a laboratory assistant.

Isolation of the organism by culture is, of course, diagnostic but requires proper equipment and culture and, it may be added, skill and experience.

At least one month's incubation is advisable before a negative culture report can be made.

POLIOMYELITIS, Prophylactic Use of Parents' Whole Blood, Stokes, J., Wolman, I. J., Carpenter, H. C., and Margolis, J. Am. J. Dis. Child. 50: 581, 1935.

The results of prophylactic injection of parents' blood, pooled convalescent serum and pooled adult serum in 2,179 children in Philadelphia have been tabulated and discussed. An attempt to analyze statistically the incidence of poliomyelitis in a group of 620 children who received injections in a certain census tract area of Philadelphia and its relation to the group who did not receive injections in the same area has been described.

A small group of 11 children who contracted poliomyelitis from five to eighteen days following the injection of whole blood or serum has been described. The mild nature of the attack was emphasized as apparently favoring such prophylaxis developed in only one child.

Neutralization tests on the serum of the convalescent children and their donors have been described. The low neutralizing power of the donor's serum in the case of the only child

who suffered from residual paralysis may be of some significance. A comparison of the results of neutralization tests on the serum of a physician and a nurse intimately exposed to the disease has been discussed.

In view of the favorable results in Philadelphia and Bradford, Pa., a further trial of the injection of parents' whole blood is recommended as a prophylactic measure. The possibility that human lyophile antipoliomyelitis serum may eliminate the present limitations of parents' whole blood has been discussed.

DICK TEST, Variability in the Strength of Toxin Used For, Friedman, E., Esserman, A. L., and Ginsburg, M. M. J. A. M. A. **105**: 956, 1935.

There is too great a variability in potency of the Dick toxin available at present, to make it serve as a dependable index to the presence or absence of immunity to scarlet fever.

Until this toxin is properly standardized and all commercial toxins are of uniform strength, it is impossible to determine with any degree of accuracy to what extent attempts at active immunization have been successful.

It would appear, therefore, that the present scheme of active immunization against scarlet fever rests on an insecure foundation.

BLOOD PLATELETS, New Method of Staining, Gradwohl, R. B. J. J. A. M. A. **105**: 1030, 1935.

Reagents:

Fourteen per cent magnesium sulphate solution. Dissolve 14 gm. of chemically pure magnesium sulphate in enough distilled water to make a total of 100 c.c. Filter through filter paper.

Methyl alcohol (National Anilin Co.).

Neutral distilled water.

For testing of neutral distilled water the following method is advised:

Pick up a few crystals of hematoxylin with a pair of metal forceps and place in a glass test tube. The tube should be neutral and contain no traces of acid or alkalis.

Add about 5 c.c. of the distilled water to be tested to the hematoxylin crystals.

The water will turn yellow, pink, or a deep purple.

If it turns to a pink color between two and five minutes, the water is neutral.

If it becomes pink before one or two minutes, the water is alkaline.

If it remains yellow for five minutes or longer, it is acid.

In neutralizing acid water, use 1 per cent sodium or potassium carbonate solution.

In neutralizing alkaline water, use 1 per cent hydrochloric or acetic acid solution.

The neutralizing agent is added to the water a drop at a time; the mixture is shaken after the addition of each drop, and then the water is tested with hematoxylin crystals.

About 2 liters of water may be neutralized at a time.

It is well to test water each day, since sometimes it becomes acid or alkaline on standing.

Fresh distilled water should be prepared every few days to insure good results.

Rubber connections on the water bottle should not be used, since the rubber from the tubing causes changes in the water that interfere with the staining.

Method:

Immerse the patient's hand in a pan of hot water to produce hyperemia. Wipe the skin dry with a clean piece of sterile gauze. Place 1 drop of magnesium sulphate solution on the end of the finger and puncture through the drop. Allow the blood to ooze through the magnesium sulphate solution until there is a mixture of about 1 part of blood and 9 parts of magnesium sulphate. Mix with the needle point. Pick up a drop of the mixture and place on a fat-free slide. Smear with a cover glass as for a blood film. The cover glass should be narrower than the slide so as to obtain margin-free smears. Fix in methyl alcohol for five minutes (in a Koplin jar).

Wash thoroughly in neutral distilled water to remove any remaining traces of magnesium sulphate solution.

Prepare diluted Giemsa stain by adding 5 drops of Giemsa stain to 5 c.c. of neutral distilled water, shaking slightly after the addition of each drop. Do not shake too vigorously.

Flood the slide with the stain on a staining bridge for thirty minutes.

Wash in neutral distilled water.

Flood the slide with dilute fresh Giemsa stain and restain for thirty minutes.

Wash in tap water, dry and examine.

Method of Making Count:

Count all the erythrocytes and blood platelets in consecutive fields until 250 erythrocytes have been counted. Count four such fields after the four-field meander method.

Calculate the number of blood platelets in 1 c.mm. of blood by the following formula:

$$\frac{\text{Patient's red count}}{1{,}000} \times \text{number of blood platelets counted} = \text{number of blood platelets in 1 c.mm. of blood.}$$

Example:

One thousand red blood cells are counted. Sixty-five blood platelets are counted. Patient's erythrocyte count, 5,000,000.

$$\frac{5{,}000{,}000 \times 65}{1{,}000} = 325{,}000 \text{ blood platelets in 1 c.mm. of blood.}$$

Normal, from 250,000 to 300,000 per c.mm.

POLIOMYELITIS, Vaccine Treatment of Acute Anterior, Kolmer, J. A., and Rule, A. M.
Am. J. Clin. Path. 5: 349, 1935.

Further evidence of the rapidity of production of immunity in monkeys by subcutaneous injections of the Kolmer vaccine of 4 per cent remote monkey passage poliomyelitis virus in 1 per cent solutions of sodium ricinoleate has been obtained.

Five of a series of ten monkeys given three subcutaneous injections of the vaccine during the incubation period one, three, and five days after intracerebral inoculation with five to ten minimal infective doses of viruses, escaped paralysis and remained perfectly well.

All of four unvaccinated control monkeys developed paralysis in from seven to eight days after intracerebral inoculation of virus.

One monkey given three doses of the vaccine on the eight day after intracerebral infection and presenting paralysis of one arm showed no further progression of the disease.

All of three monkeys given three doses of the vaccine two to five days after the onset of paralysis were not improved and developed progressive paralysis.

The results have indicated the possibility of the vaccine's effectively protecting some monkeys against experimental poliomyelitis when given during the incubation period of the disease.

FLUIDS, Pathological Body, Cholesterol Partition and Total Protein Content, Bruger, M.
Am. J. Clin. Path. 5: 384, 1935.

In pathologic body fluids, the total cholesterol parallels the total protein content; exudates are characterized by high cholesterol and high protein contents and transudates (including nephrotic edema fluid) by low cholesterol and low protein contents.

The total cholesterol content in pathological effusions bears no direct relation to the total cholesterol content in the plasma. The concentration of this sterol in effusates may approach, but with rare exceptions does not exceed, that in the plasma.

The relation of free to ester cholesterol is alike, at times, in pathological body fluid and in plasma; at other times, the cholesterol partition in effusate and plasma differ markedly.

A serial study of the pleural effusions in a patient with adenocarcinoma of the lung indicates to what extent the human organism can be deprived of cholesterol by abnormal secretions; this patient lost more than 12 gm. of cholesterol in eight liters of pleural fluid.

The Journal of Laboratory and Clinical Medicine

VOL. 22 JULY, 1937 NO. 10

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EDITORIAL

State Licensure of Medical Technologists

IN THIS day of regimentation, licensure, federal and state molding, it is not surprising that a movement should be started to license medical technologists. The matter has been given serious consideration by the Executive Committee of the American Society of Clinical Pathologists, the Board of Registry and the Council on Medical Education and Hospitals of the American Medical Association. These groups are decidedly opposed to the enaction of such legislation because there is already provided by the Board of Registry a clearinghouse for the certification of medical technologists. This saves the technician state fees and makes in one central place a record of all qualified technologists. This certification is free of political pressure and other abuses which have all too frequently appeared in state-regulated professions. The present Registry certification eliminates duplication, waste effort, and unnecessary legislation.

The qualifications required of technologists are uniform in all parts of the country under the present registry, eliminating partiality toward or

prejudice against any region or state. The scheme as established by the Board of Registry has been approved by the American Medical Association, American College of Surgeons, and the American Hospital Association.

It is quite likely that the granting of state licenses to practice medical technology will permit technologists to set up their own laboratories and thus under such conditions they will be practicing medicine. This is perhaps the most serious aspect of the whole situation. It has been clearly established that the practice of clinical pathology is an essential specialty in medicine and that it should be practiced only by licensed, trained, and competent medical men. To permit technicians to practice clinical pathology without the supervision of such trained physicians would obviously be to the detriment of the patient, who deserves the first consideration. The whole subject of the relation of the medical technician to the patient and to the physician is not completely settled, but state legislation will do well to permit those physicians who are giving their lives to this particular subject to work out the proper solution of the problem. At the present time the relation of the technician to the practice of medicine is being fairly well handled and as more experience is acquired by the Board of Registry it undoubtedly will be able to make a completely satisfactory solution of the whole problem.

—T. B. M.